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The Diagnostic Utility of RT-QuIC for the Diagnosis of Sporadic Creutzfeldt-Jakob Disease

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Declaration of Authorship

I hereby confirm that this thesis was composed by myself, and is the product of my own work. Specific aspects of this work were undertaken in collaboration with colleagues at the National CJD Research and Surveillance Unit, and this is acknowledged in the text. This work has not been submitted for any other degree or professional qualification.

Dedication

This thesis is dedicated to Mark, Louise, Thomas and Claudia. Thank you for all of your love, support and encouragement.

Acknowledgements

I would like to thank all of the staff at the National CJD Research and Surveillance Unit (NCJDRSU) for their patience, kindness and support. In particular, I would like to thank my research supervisors Professor Richard Knight and Professor Robert Will whose guidance has been invaluable, not only during my role as research registrar and production of this thesis but also as a neurologist. I would also like to thank my friend and colleague Dr Alison Green, who has been a phenomenal support both professionally and personally. In addition, thank you to Miss Jan Mackenzie who just seems to know everything you need to know at the time you need to know it.

Finally, I would like to thank the contributions of patients, their families and clinicians, without whom, this work would not have been possible, and the bodies funding the NCJDRSU.

Abstract

Introduction

Sporadic Creutzfeldt-Jakob Disease (sCJD) remains the commonest type of prion disease with an incidence of approximately 1 per million and accounting for approximately 80% of cases. The exact infectious mechanism is unknown. However, the most widely accepted theory is the 'protein-only hypothesis' initially suggested by Prusiner and later developed over the years. It has been proposed that prion diseases result from the post translational change of a normally expressed protein (PrP^{C}) into a disease associated form (PrP^{Sc}). PrP^{Sc} is partially protease resistant and reserves the ability to self-propagate by inducing further PrP^{C} to undergo conformational change thus producing more PrP^{Sc}, a so called 'seeding' effect. PrP^{Sc} subsequently aggregates throughout the brain, producing the neuro-pathological hall mark of prion diseases which includes spongiform change, neuronal loss and astrocytic gliosis.

Despite its rarity, the clinical presentation is well described and classically follows a characteristic course of rapid onset dementia with associated neurological decline that often includes cerebellar ataxia, myoclonus, eventually leading to a state of akinetic mutism and death within a period of 4-6 months. Atypical presentations are less common but are well documented in the literature.

Currently, there is not a disease specific ante-mortem test available for the diagnosis of sCJD with post mortem remaining the definitive means of diagnosis. Current diagnostic criteria rely on clinical presentation in association with the MRI, EEG and CSF 14-3-3 protein. However, none of these investigations are specific for sCJD. Atypical presentations can be diagnostically challenging and there can be a delay in diagnosis which can be distressing for relatives. This identified a need for a disease-specific, reliable diagnostic test that can provide an earlier and more accurate diagnosis.

A recently developed assay called real time quaking induced conversion (RT-QuIC) exploits the seeded conversion of normal prion protein to the abnormal form and therefore detects disease-associated prion protein in the cerebrospinal fluid. Based on recent evidence, it has RT-QuIC has been reported to be highly sensitive and specific for diagnosing sporadic CJD and has the potential to identify cases that conventional diagnostic techniques such as electroencephalogram and MRI may miss, potentially contributing to an earlier and more accurate diagnosis. This study aimed to provide a prospective analysis of the utility of RT-QuIC in routine clinical practice.

Aims of Thesis

The aims of this work were:

- To prospectively assess the diagnostic utility of RT-QuIC in routine clinical practice
- To assess if certain clinical factors affect the RT-QuIC result, for example, codon 129 genotype, age at onset, symptoms at onset and duration of disease.
- To determine the value of RT-QuIC in cases where there is diagnostic uncertainty using current diagnostic techniques and establish whether it can provide an earlier diagnosis
- To review the operational parameters (for example CSF volume, timing of lumbar puncture) of RT-QuIC throughout the course of the study and contribute to the optimisation and development of this new diagnostic test.

Methods

162 suspected cases of sCJD were referred to the National CJD Research and Surveillance Unit (NCJDRSU) within the 18 month study period. 146 suspected cases underwent a lumbar puncture and 115 of the clinically suspected cases had 14-3-3 and RT-QuIC analysis performed. All cases were examined by the author where possible and the family were interviewed using a standardised questionnaire. All cases were classified according to the current EuroCJD criteria pre and post review. MRI and EEG were reviewed where possible. Genetic testing and codon 129 were analysed following obtained consent. Brain tissue following post mortem examination was also reviewed where possible.

Results

44 cases were classified as pathologically confirmed sCJD. Of these, 35 (79.9%) were RT-QuIC positive. 36 (81.8%) were 14-3-3 positive, 14 (31.8%) were EEG positive and 24 (54.5%) were MRI positive. 45 cases were classified as 'probable' according to WHO criteria, 38 (84.4%) were RT-QuIC positive, 40 (88.8%) were 14-3-3 positive, 14 (31.1%) were EEG positive and 27 (60.1%) were MRI positive. There were 5 cases classified as 'possible' sCJD of which 3(60%) were RT-QuIC, 0 were 14-3-3, MRI or EEG positive. 7 cases were classified as 'unknown' cases of sCJD of which 2(28.6%) were positive for both RT-QuIC and 14-3-3. 3(42.8%) had a positive MRI and 0 cases had a positive EEG.

Overall, RT-QuIC has a sensitivity of 80% and specificity of 100% with a positive predictive value of 100%. In comparison, 14-3-3 has a sensitivity of 82%, specificity of 82% and PPV of 95%. EEG has a sensitivity of 32%, specificity of 91% and PPV of 93%. MRI has a sensitivity of 55%, specificity of 100% and PPV of 100%.

During the initial data period, 15µl of CSF was used to perform the RT-QuIC test although over the course of the study, it was found that 30µl gave better discrimination between positive and negative RT-QuIC results. However, some CSF samples from cases of

suspected sporadic CJD had a negative RT-QuIC response at 30µl but a postive RT-QuIC response was obtained using 15µl. Therefore, CSF samples were tested at both 15µl and 30µl.

Discussion

In concordance with current literature, RT-QuIC is similar in sensitivity to 14-3-3 but more specific and has a greater positive predictive value. It is considerably more sensitive than EEG and more specific. It is more sensitive than MRI but has equal specificity and positive predictive value. The 3 cases classified as 'possible' and RT-QuIC positive were highly suspected to be cases but died without a post-mortem. The 2 cases classified as 'unknown' and RT-QuIC positive were both clinically suspected to be cases but also died without a post-mortem. However, since this study was started, based on further European and International research on RT-QuIC, the European diagnostic criteria for the diagnosis of sCJD has changed as of January 2017 to include RT-QuIC. Therefore, based on the new criteria, all of these cases would now be considered likely cases of sCJD. Overall, this is a highly sensitive, specific and reliable test for the diagnosis of sCJD, especially in cases where the diagnosis is difficult and when conventional tests may fail. This study supports the current literature.

Lay Summary

Introduction

Sporadic Creutzfeldt-Jakob Disease (sCJD) is a rare brain condition with an incidence of 1 per million per annum. It presents with a recognisable pattern of symptoms with rapidly progressive dementia frequently being a key feature. The condition is unequivocably fatal and death usually ensues within 4-6 months of symptom onset. The cause of sCJD remains unknown. The most widely accepted theory is based on a specific type of protein that we all have in our brains called the prion protein. Its exact function remains elusive although it is thought to play a part in our brains being able to function normally. The prion protein is folded into a specific shape and this shape is important for it to work normally. It has been hypothesised that in sCJD, this protein unfolds into a different shape and therefore is unable to work normally. In addition, the abnormally folded protein then triggers other prion proteins to do the same, almost like a domino effect. This produces very characteristic changes in the brain tissue which can be seen under a microscope following post mortem. Post mortem remains the definitive means of diagnosing the condition. In life, there is no perfect test to make the diagnosis. Diagnostic criteria was introduced and developed over the years and include the presence of a recognisable pattern of symptoms in addition to suggestive abnormalities on MRI brain scanning, specific abnormalities on a brain wave test (called and encephalogram or EEG) and the presence of protein in the fluid that bathes the brain (called 14-3-3). This is a different type of protein that the brain releases into the spinal fluid whenever there is a disease process affecting the brain.

The difficulty with current tests is that they are not specific for sCJD and the changes observed on these tests can be seen in other neurological conditions that can affect the brain. In addition, atypical presentations of sCJD are well recognised which the conventional tests may miss. This identified a need for a more disease specific test that was reliable and more accurate. Over recent years a new type of spinal fluid test was introduced called real time quaking induced conversion or RT-QuIC for short. In this test, a synthetic form of prion protein is added to the spinal fluid of a person suspected of having sCJD. If the abnormal protein is present, it should start to convert the normal prion protein to an abnormal form.

Using a special fluorescent marker, this process can be monitored and gives rise to a positive result. If there is no fluorescent effect then the test is negative. Current evidence has indicated that RT-QuIC can diagnose sCJD with higher accuracy and potentially identify cases that conventional tests may miss. This study aims to assess the use of RT-QuIC in routine clinical practice.

Aims of thesis

The aims of this work was:

- To assess the use of RT-QuIC in routine clinical practice
- To identify whether any clinical factors such as age at onset, disease duration, underlying genetics influence the outcome of the test.
- To ascertain whether RT-QuIC can diagnose atypical presentations that the conventional tests may miss
- To contribute to the development of the test throughout the course of the study. For example, what is the best volume of spinal fluid to use when completing the test

Methodology

The National CJD Research and Surveillance Unit (NCJDRSU) was established in 1990. We rely on the referral of clinically suspected cases of CJD by clinicians from throughout the United Kingdom. As part of surveillance, the unit has a number of other roles including analysis of 14-3-3 and RT-QuIC, review of MRI and EEG, genetic analysis and neuro-pathological examination of brain tissue. Suspected cases are offered a visit by a doctor from the unit. In addition to patient assessment, surveillance data is obtained during an interview with families using a standardised questionnaire.

162 suspected cases of sCJD were referred to the NCJDRSU within the 18 month study period. 146 suspected cases underwent a lumbar puncture and 115 of the clinically suspected cases had 14-3-3 and RT-QuIC analysis performed. All cases were examined by the author where possible and the family were interviewed using a standardised questionnaire. All cases were classified according to the current WHO criteria pre and post review. MRI and EEG were reviewed where possible. Genetic testing was performed following obtained consent. Brain tissue following post mortem examination was also reviewed where possible.

Results

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Overall, RT-QuIC has a sensitivity of 80% (the percentage of cases that were correctly identified as positive) and specificity of 100% (the percentage of cases that were correctly identified as negative). In comparison, 14-3-3 has a sensitivity of 82% and specificity of 82%. EEG has a sensitivity of 32% and specificity of 91%. MRI has a sensitivity of 55%, specificity of 100% and PPV of 100%.

Clinical factors including disease duration, age at onset, timing of lumbar puncture did not appear to influence the RT-QuIC result.

During the initial data period, 15 microlitres of CSF was used to perform the RT-QuIC test although over the course of the study, it was found that 30 microlitres was better at

differentiating between positive and negative RT-QuIC results. However, some CSF samples from cases of suspected sporadic CJD had a negative RT-QuIC response at 30 microlitres but a postive RT-QuIC response was obtained using 15microlitres. Therefore, CSF samples were tested at both 15microlitres and 30microlitres.

Discussion

In concordance with current literature, RT-QuIC is similar in sensitivity to 14-3-3 but more specific. It is considerably more sensitive than EEG and more specific. It is more sensitive than MRI but has equal specificity. The 3 cases classified as 'possible' and RT-QuIC positive were highly suspected to be cases but died without a post-mortem. The 2 cases classified as 'unknown' and RT-QuIC positive were both clinically suspected to be cases but also died without a post-mortem. However, since this study was started, based on further European and International research on RT-QuIC, the European diagnostic criteria for the diagnosis of sCJD has changed as of January 2017 to include RT-QuIC. Therefore, based on the new criteria, all of these cases would now be considered likely cases of sCJD. Overall, this is a highly sensitive, specific and reliable test for the diagnosis of sCJD, especially in cases where the diagnosis is difficult and when conventional tests may fail. This study supports the recent literature.

Chapter 1

Introduction

Introduction

1.1 Prion Diseases: an overview

Creutzfeldt-Jakob Disease (CJD) belongs to a group of fatal neurodegenerative diseases collectively known as Prion Disease or Transmissible Spongiform Encephalopathies (TSEs). Prion diseases affect both animals and humans and can occur in sporadic, acquired or inherited forms. They are characterised by the fact that they are potentially transmissible and by their recognisable neuro-pathological and molecular features.

The existence of acquired forms of prion disease and transmissibility support the presence of an infectious agent[1]. However, the exact infectious mechanism remains unknown. The most widely accepted theory is the 'protein-only hypothesis', initially suggested by Prusiner and later developed over the years[2]. It has been proposed that prion diseases result from the post-translational change of a normally expressed protein, named the prion protein (PrP^{C}) into a disease associated form (PrP^{Sc})[2,3]. PrP^{C} is found in abundance in many tissues throughout the body, although predominantly in the central nervous system[4]. Its exact function remains unclear. It is not thought to be essential for life although, interestingly, studies on transgenic mice have demonstrated that PrP^{C} is essential for the transmission of prion disease[5]. PrP^{Sc} is partially protease resistant and has the ability to self-propagate by inducing further PrP^{C} to undergo conformational change thus producing more PrP^{Sc}, a so called 'seeding' effect[5]. PrP^{Sc} subsequently aggregates and is deposited throughout the brain producing the neuro-pathological hallmark of prion diseases along with spongiform change within the brain, along with neuronal loss and astrocytic gliosis[6].

Historically, epidemics of CJD have arisen in humans via the cannabalistic consumption of infectious body tissues during funerary practices in Papa New Guinea (termed Kuru) and more recently in the form of Variant CJD (vCJD)[7,8]. Variant CJD is a zoonosis that developed following the contamination of the human food chain by Bovine Spongiform Encephalopathy (BSE), a TSE affecting cattle[9,10].

In addition, secondary transmission of vCJD by blood transfusion and the use of a blood product have also been reported[11-16]. Iatrogenic CJD (iCJD) is the consequence of unintended transmission of CJD via certain medical/surgical procedures involving infectious human material or contaminated instruments[17]. Genetic prion diseases were initially described over a hundred years ago, when neurodegenerative diseases characterised by ataxia and dementia were observed in families across several generations[18-20]. It is now known that this is due to mutations of the gene which encodes the normal cellular prion protein PrP^{C} (*PRNP*). Although autosomal dominant in inheritance, cases of genetic disease have been reported in the absence of a family history of the condition[21].

Currently, the commonest form of prion disease is CJD with sporadic CJD (sCJD) accounting for ˜80% of cases with an annual mortality rate of approximately 1-2 per million of the population in most countries[22]. The exact aetiology of sCJD remains unknown. Despite its rarity, the clinical presentation has been well described with rapidly progressive dementia being a central feature. Death typically ensues within 4-6 month of symptom onset[23, 24]. However, clinical heterogeneity is well recognised in sCJD resulting in challenging and sometimes delayed diagnosis[25, 26]. Early and accurate diagnosis of CJD is imperative for three reasons. The differential diagnosis of rapidly progressive dementia is wide and diagnosing or excluding CJD early may negate the need for unnecessary investigations or empirical treatment[25, 27]. Secondly, there are the obvious public health implications and prevention of spreading the disease via iatrogenic means[28]. Lastly, an earlier diagnosis has the potential to ease the distress of the families of patients suffering from the condition. In addition, if treatments are developed, early diagnosis would allow early treatment, with potentially better therapeutic outcome. Having spoken at the CJD Support Network charity conferences in 2013 and 2014, I learned that providing an earlier diagnosis, as well as identifying a treatment, were considered the main aspects of future research that were a priority to many families who have lost relatives to the condition.

The early and accurate diagnosis has been a longstanding problem with standard diagnostic techniques lacking in specificity and not accounting for the clinical

heterogeneity of the condition[25]. Here, I will provide a summary of prion diseases, concentrating on sCJD, the standard methods of diagnosis, their limitations and the emergence of newer, more disease specific diagnostic techniques.

1.2 The Prion Protein and Protein-Only Hypothesis

PrP^{C} is the normal cellular form of PrP^{Sc}, the molecular hallmark of prion disease. The gene encoding this protein in humans is *PRNP*, located on chromosome 20. In adults, PrP^{C} is highly expressed in the central nervous system (commonly located on the neuronal cell membrane) although its exact function remains unclear but it is thought to play a role in the development of the nervous system[21, 25].

The normal cellular protein is a glycophosphatidylinositol-anchored (GPI) glycoprotein with a largely α-helical C-terminal domain and an intrinsically disorganised N-terminal domain that binds copper and zinc[21, 25, 29]. The majority of PrP^{C} is produced in the endoplasmic reticulum and GOLGI apparatus. Thereafter it migrates to the cell surface where it is typically located attached by the GPI anchor and exposed to extracellular contents. However, during its lifecycle, PrP^{C} is internalised into endosomes and then re-cycled to the cell surface again[25, 29]. PrP^{C} can also be cleaved internally by endogenous proteases to produce N-terminal and C-terminal fragments[25].

PrP^{C} is expressed in both the central nervous system and peripheral tissues, although it is found in abundance within neurons[25]. Describing the role of PrP^{C} in healthy individuals has remained elusive. Manipulation of PrP^{C} levels has been reported to alter a number of cellular functions including metal homeostasis, maintenance of the peripheral nerve myelin, synaptic plasticity, neuro-protection and cell signalling[30-36].

Prion proteins have become well known for their causative role in a range of neurodegenerative diseases in humans and animals. Human prion diseases include sCJD, Gerstmann Sträussler-Scheinker disease (GSS), Familial Fatal Insomnia (FFI), genetic CJD (gCJD), Kuru, Variably Protease Sensitive Prionopathy (VPSPr) and vCJD. Animal prion diseases include Scrapie in sheep, goats and mouflon,

Transmissible Mink Encephalopathy (TME), Chronic Wasting Disease (CWD) in deer and Bovine Spongiform Encephalopathy (BSE) in cattle. Over the years, extensive research has been conducted in attempt to establish the types of prion diseases and their infectious mechanisms.

Prion diseases share a number of common histopathological characteristics and neurological symptoms. These include spongiform degeneration of the central nervous system, formation of amyloid plaques, reactive gliosis and neuronal loss[6, 37-39]. When prion diseases arise as infections, they have a number of notable or atypical features: potentially long incubation periods, a lack of inflammation response and a lack of disease specific immune response[40]. Initially these diseases were thought to be caused by a 'slow virus' due to their long incubation periods. However, neither a virus nor nucleic acids have been detected to support this theory. In addition, the 'viral hypothesis' fails to account for the fact that up to 10-15% of cases are dominantly inherited genetic diseases and only a relative minority of cases (iatrogenic and variant CJD) have a clear infectious cause[40-42].

The most widely accepted theory regarding the infectious mechanism of prion diseases is the 'protein-only hypothesis' initially proposed by Griffith[2], later developed over the years and now supported by a compelling body of evidence[40, 43-46]. Prusiner postulated that the infectious agent was not a virus but a 'novel infectious entity' consistent of protein alone and coined the phrase 'prion' to demonstrate the *pro*teinaceous and *in*fectious nature of the agent[3]. This hypothesis suggests that prion diseases result from a post-translational conversion of the normal prion protein, PrP^{C}, to a misfolded form, PrP^{Sc}. This newly formed abnormal protein then acts as a template to facilitate conversion of further PrP^{C} to PrP^{Sc} leading to further accumulation and aggregation within the central nervous system and ultimately resulting in disease[3, 40, 43, 44, 45]. Of interest is that this process occurs regardless of the origin of PrP^{Sc} [39]. For example, it will occur if the abnormal prion protein is acquired through an external source, as seen in vCJD. Alternatively, the source can be internal due to mutations in the *PRNP* gene as in GSS, FFI and genetic CJD. In addition, it will also occur in cases of spontaneous conversion of PrP^{C} or

somatic mutation of *PRNP* as have been suggested as potential mechanisms in sporadic CJD [42, 47].

Studies have demonstrated that the PrP^{Sc} is characterised not only by its misfolded shape with a high β sheet content, but also the fact that, unlike PrP^{C}, it is insoluble and partially resistant to protease digestion[40, 45, 48, 49] . In contrast, PrP^{C} is a soluble protein high in α helix structure, low in β sheet content and highly susceptible to protease degradation[40, 45]. This transition from predominantly α-helical to β sheet content appears to be a central event in prion propagation and pathogenesis although the exact mode by which it occurs remains unknown. In prion disease, most, if not all, the α-helical structure is refolded into β sheets and the abnormal prion protein forms oligomers and then multimers that can then form amyloid fibrils[50]. Some researchers believe that it is the accumulation of PrP^{Sc} that results in the neurodegeneration within the brain[50]. However, other groups believe that it is the process of conversion from PrP^{C} to PrP^{Sc} that causes the neuronal injury and cell loss[51, 52].

The accumulation of PrP^{Sc} in the central nervous system is a feature that is common to all prion diseases. Studies have shown that the accumulation of PrP^{Sc} is a relatively late event which occurs shortly before the initial clinical symptoms and signs develop[53, 54]. In variant CJD, but not other human prion disease, peripheral lymphoid tissues such as tonsil, appendix, spleen and lymph nodes show abnormal prion protein accumulation well before brain involvement and clinical signs are manifest[55]. This obviously has public health implications where there is potential for transmission.

1.3 Prion Disease in Animals

The main forms of animal prion disease include Scrapie in sheep, transmissible mink encephalopathy (TME), chronic wasting disease in cervids (CWD) and bovine spongiform encephalopathy (BSE) of cattle. Some animal prion diseases have been transmitted through contaminated feed or via a contaminated environment[56, 57].

1.3.1 Scrapie

Scrapie was the first TSE described and is considered the prototypic TSE. The first reported case of this neurodegenerative prion disorder affecting sheep, mouflon and goats occurred in the United Kingdom in 1732[57]. The official name of Scrapie was used from 1853 and was derived from one of the principle signs of the condition where the animal compulsively scrapes their fleece against a hard surface due to an intense sensation of itching, frequently leading to wool loss[57]. Classical and atypical forms of the condition are now recognised. Other clinical signs of the classical form include behavioural change (head tremor, teeth grinding, hyper-responsive, agitation), increased thirst, excessive lip smacking, altered gait, incoordination and convulsions[58]. In atypical forms of the condition, pruritis is usually absent and ataxia and incoordination are more pronounced[59, 60]. It is a naturally occurring infectious disease with an estimated incubation time of 2-5 years and death usually ensues within 2 weeks to 6 months from the symptomatic onset[61].

Contamination of the environment was initially attributed to infectivity in the placenta from scrapie infected ewes[62]. However, other sources of environmental contamination have now been established including urine, faeces and saliva[63-65]. This would explain cases of Scrapie where lambing had not occurred[66, 67]. Scrapie PrP^{Sc} has been detected in a number of peripheral tissues including tonsils, spleen, lymph nodes, muscle and colon[68]. Laboratory studies have demonstrated that PrP^{Sc} can attach to soil particles that remain near to the surface and are therefore accessible to grazing animals implicating soil as a plausible reservoir for Scrapie infectivity[69].

In addition, abnormal prion PrP^{Sc} is able to persist in soil for years without losing its infectious properties[70]. For example, there have been reports of sheep being infected in a building that had been unused for 16 years[71].

Detectable PrP^{Sc} has been reported in the faeces of sheep both in the early preclinical and end stages of the disease, suggesting that prions are likely to be shed into the environment throughout the course of the disease[64]. It is invariably fatal and contagious resulting in the disease becoming endemic within flocks and remains a notifiable disease. It is not thought to pose a threat to humans[72].

1.3.2 Transmissible Mink Encephalopathy (TME)

TME is rare and affects ranch raised mink and is thought to have been acquired through contaminated feed although the exact origin of the infectious agent is unknown[61]. Four outbreaks of TME were reported in the United States between 1947 and 1985 and no cases have been documented since this time[73, 22]. Many of the cases occurred in Wisconsin but ranches in Minnesota and Idaho were also affected[73]. TME has also been reported in ranch raised mink in Canada, Finland, Germany and Russia[61, 73]. The incubation period is thought to be approximately 6-12 months with clinical onset to death usually between 2-8 weeks[74]. The source of these outbreaks is unclear although epidemiological studies have suggested that Scrapie contaminated feed is the most plausible cause[74].

The clinical phenotype is usually a gradual onset of behavioural change with increased aggression and hyperaesthesia, abnormal gait, compulsive biting, blindness and coma[61]. Whether TME can survive long term in the environment remains unclear, but unlike Scrapie, TME does not seem to recur during subsequent years on the same farm. However, like Scrapie, TME is contagious and outbreaks invariably lead to death of an entire herd[74].

1.3.3 Chronic Wasting Disease (CWD)

CWD is the only known prion disorder affecting free ranging wildlife including mule deer, white tailed deer, black tailed deer, elk and moose[75]. It was initially recognised as a clinical 'wasting' syndrome in 1967 in captive mule deer in Colorado, USA. It was later identified as a TSE by Williams in 1980 and has spread to free-range and captive populations in 23 US states and 2 Canadian provinces[76, 77, 78]. Following exportation of farmed cervids, cases were also detected in the Republic of Korea[79, 80]. In 2016, the first case of CWD in Europe was discovered in free ranging reindeer in Norway[81].

CWD is highly infectious and readily transmitted amongst cervids. They continue to expand in prevalence and geographical range in North America since its initial discovery almost 50 years ago: it is considered the most contagious prion disease[82]. Presently, there is significant variation in the prevalence of CWD throughout North America, with levels ranging from 0-30% in wild populations[83] and approaching 80% in captive groups[84].

Factors that influence the variation in prevalence and geographical spread, remain incompletely understood. The most convincing and leading hypothesis for the progressive spread of the condition in wild populations is the presence of CWD prions in bodily fluids[75, 85]. CWD prions has been identified in saliva, blood, faeces and urine in transgenic mice expressing cervid PrP^{C} with oral and nasal inoculation proving an efficient means of transmission[85]. Transmission is therefore thought to be facilitated through exchange of bodily fluids between animals and the shedding of PrP^{Sc} into the environment and subsequent indirect transmission through ingestion of contaminated water, soil and plants[85].

The clinical hallmark of CWD is chronic weight loss and emaciation leading to death. Other signs include behavioural changes including decreased interaction with other animals and loss of fear to humans, weakness, excessive salivation and bruxism, polydipsia, polyuria and tremors[86].

Currently, there has been no evidence of transmission to humans but ongoing research and surveillance continues in order to assess the zoonotic potential. Transmission studies of CWD prions to humanised transgenic mice or non-human primates suggest a strong species barrier[87]. However, in vitro studies have demonstrated that human PrP can be converted by CWD prions into PrP^{Sc} upon adaptation[88]. Therefore the potential for zoonotic transmission cannot be fully excluded. CWD is not transmissible to mice expressing human PrP^{C} although it is efficiently transmitted to mice expressing cervid PrP^{C} [75]. It is thought that this difference is likely to reflect a species barrier, at least in part, likely to be the consequence of differences in amino acid sequences of the host cellular prion protein between species, thus demonstrating the importance of amino acid sequence in disease susceptibility[75]. A recent systematic review investigating the current evidence of the transmissibility of CWD prions to humans found that five epidemiological studies, two studies on macaques and seven studies on humanised transgenic mice did not provide evidence to support transmission to humans[89]. Further work has been done by Race et al who also used cynomolgus macaque monkeys as a surrogate model for CWD transmission to humans[90]. Following inoculation, a variety of highly sensitive disease screening assays were used to assess the presence of abnormal prion protein over a 13 year clinical observation period. There was no evidence of CWD transmission in the specimens examined[90]. However, there have been two studies on squirrel monkeys as well as several in vitro experiments that provided evidence that CWD prions can seed human prion protein to a misfolded state. It was therefore concluded that transmission of CWD prions to humans cannot be ruled out as a possibility and therefore ongoing research and surveillance is important, particularly given the potential for long incubation periods[89].

1.3.4 Bovine Spongiform Encephalopathy (BSE)

Bovine spongiform encephalopathy (BSE) is a transmissible spongiform encephalopathy that occurs in cattle, initially recognised in the United Kingdom in 1985[91]. BSE has a long incubation period of 2.5-5 years and usually affects cattle of adult age. Clinically it is characterised by an abnormal gait, paralysis of the hind legs, changes in behaviour, tremors and hyper-responsiveness to stimuli. Other non-specific symptoms include weight loss, decreased milk production and pacing. Death usually ensues within 2 weeks to 6 months from the development of symptoms[92].

BSE was most likely rose from infectivity in meat and bone meal (MBM) made from slaughtered cows and sheep as cattle feed. From 1972, In the UK, cow intestines were used in MBM as a protein supplement which accelerated the occurrence of BSE[93]. BSE has occurred in European countries that import MBM from the UK, however, most reported cases are from the UK, peaking in 1992[57].

The original source of the infectious material was at first suspected to be scrapie sheep but the possibility of a spontaneous bovine prion disease has not been excluded[57].

The compulsory notification and slaughter of cases of BSE (over 3 million cows) and a ban on MBM use was effective in controlling the epidemic[57, 94]. Initial studies suggested that isolates from cases of BSE were homogeneous. However, the possibility of a mixture of strains within BSE was later suggested and subsequent studies have provided strong evidence that this is the case. Rarer variants of BSE have now been identified with isolates of PrP^{Sc} demonstrating profiles that differ in their biochemical and molecular properties. Western Blot analyses identified types of PrP^{Sc} that had a different pattern of unglycosylated band migration compared to the classic form of BSE, known as ‘C type’. Profiles that migrated to a higher (H Type) or lower (L Type) position were demonstrated. A further study by Jacob et al also noted differences within molecular mass, antibody affinity binding, proteinase kinase digestion thus providing a strong argument for the presence of different

strains of BSE. Histological features of the initial L type isolates also showed a proclivity for the forebrain in comparison to the classical form of BSE which has a higher density of PrP^{Sc} deposition in the brainstem[95]. In addition, the PrP^{Sc} formed unusual amyloid plaques which led to the name Bovine Amyloidotic Spongiform Encephalopathy (BASE). [96, 97].

1.4 Prion Disease in Humans

Human prion diseases currently recognised: Kuru; sCJD; vCJD; iCJD; VPsPr and the inherited forms which include GSS, FFI and genetic CJD (gCJD). Sporadic CJD accounts for over 90% of prion disease. The different forms of human prion disease share neuropathological features and the characteristic deposition of PrP^{Sc} in tissue, especially brain tissue: the identification of this remains the definitive means of diagnosis.

1.4.1 Kuru

Kuru is a prion disease that became an epidemic amongst the Fore and surrounding groups in Papa New Guinea in the late 1950s. The term Kuru was derived from the Fore word Kuria or Guria which means 'to shake'[98]. It is now widely accepted that Kuru was transmitted and propagated among members of the Fore tribe of Papa New Guinea through ritualistic mourning cannibalism[98]. Deceased family members of the tribe were traditionally cooked and eaten in order to demonstrate love and respect that was thought to help free the spirit of the dead[98]. Females and children usually consumed the brain and spinal cord, the organs in which the infectious prions were most concentrated, thus allowing the transmission of Kuru. This disease was subsequently more prevalent among women and children[98]. In 1959, these funerary practices were banned by Australian authorities and no individual born after 1959 has been diagnosed with the condition. However, the mothers of these children continued to die suggesting that vertical transmission did not occur[98, 99]. Affected women who had married into neighbouring villages were shown to have contributed to the epidemic[98]. Studies have suggested the epidemic may have started around 1900 and it has been postulated that the origin was a sporadic case of CJD[100]. This suggestion is supported by the fact that the molecular and biological properties between Kuru and sCJD are indistinguishable[101].

The disease has lingered due to Kuru's long incubation period from anywhere between 10 to over 50 years. However, the epidemic has declined from over 200 deaths per year in 1957 to no deaths in 2005[102].

The disease itself is characterised by progressive cerebellar ataxia and interestingly dementia was frequently late in the disease or did not occur at all. The preclinical phase averages between 10-13 years but can be as short as 5 or as long as 50 years after initial exposure. Symptoms start with cerebellar signs and gait ataxia, followed by severe tremors, emotional lability and later dysphagia, mutism and eventually death[102]. Histologically, amyloid plaques containing PrP^{Sc} are seen in the cerebellum, accompanied by cerebellar atrophy, generalised astrocytosis and neuronal degeneration with relatively mild spongiform change[103].

1.4.2 Creutzfeldt-Jakob Disease (CJD)

CJD is the most frequent of the human prion diseases although it remains rare. The vast majority of CJD cases are sporadic (85-90%) although 5-15% are familial and 1% iatrogenic, iCJD[104, 105]. The annual mortality rate of sporadic CJD is approximately 1 per million persons with a worldwide distribution[22]. The median age of the onset of the disease is between 57 and 62 years with an extensive age range between 14-92 years old[22]. Patients with vCJD and iCJD are usually much younger which led to an earlier appreciation that the mode of transmission might be different. Genetic CJD patients have only a slightly younger age of onset compared with sporadic CJD.

1.4.2.1 Sporadic CJD (sCJD)

Sporadic CJD (sCJD) remains the commonest type of CJD with an annual mortality rate of approximately 1-2 per million persons per year worldwide[22]. It is essentially a disease of the middle aged and elderly with a median age of onset 70 years[104], although younger cases also occur[22]. The cause of sCJD remains unknown despite

large scale epidemiological studies. Despite its rarity, the clinical presentation has been well described. The typical clinical picture consists of a rapidly progressive, short duration illness dominated by dementia with associated neurological decline that often includes myoclonus, visual symptoms and cerebellar ataxia, eventually leading to a state of akinetic mutism and death[105]. In some cases, psychiatric symptoms, behavioural change and insomnia can occur[23]. The disease progression is typically rapid with a median disease duration to death of 4.5 months[106]. The cognitive decline may manifest as a global dementia, although specific behavioural abnormalities or particular cognitive issues may be prominent in the early stages. The disease is fulminant and deterioration may be observed on a day to day basis with death ensuing sometimes in a matter of weeks[106]. Myoclonus, especially provoked by startle, is present in more than 90% of patients at some point during the illness but may be absent at presentation, even when the dementia is profound[23]. Other recognised symptoms include visual symptoms including visual blurring or loss, diplopia or visual field defects which may progress to cortical blindness. Visual hallucinations and misperceptions are also common. Extrapyramidal signs (such as hypokinesia), cerebellar and corticospinal tract involvement may also occur. The mode of death usually relates to a reduced conscious level and loss of the swallow reflex, frequently resulting in aspiration pneumonia[23, 106].

However, while almost 80% of cases follow this typical presentation, atypical cases are also recognised and often pose a diagnostic challenge. Atypical presentations are less common but clinical heterogeneity in sporadic CJD is a recognised phenomenon and can be diagnostically challenging[23, 25]. Two particularly well documented atypical forms include a predominantly cerebellar onset (Oppenheimer-Brownell variant)[107], a predominantly visual onset (Heidenhain variant)[108] and more rarely, a stroke-like onset has also been reported[109]. Cases of patients presenting with isolated aphasia and spastic paraparesis have also been reported[23]. Younger patients with sporadic CJD have clinical features that are somewhat distinct from the more typical older patient[23]. Pocchiari et al conducted a collaborative study in 2004 and assessed some of the predictors of survival in sporadic CJD. The group concluded that longer survival was associated with younger age at onset of illness and female gender, codon 129 heterozyosity (discussed below) and certain investigations results[110].

Atypical presentations of sCJD differ not only by their clinical presentation but also in disease course, diagnostic test results and specific neuropathological characteristics. This spectrum of clinico-pathological variation has been correlated with different genotypes at codon 129 of the prion protein gene (*PRNP*) on chromosome 20 and also different conformations of the abnormal prion protein, PrP^{Sc}. A clinico-pathological molecular classification of sCJD was suggested by Parchi et al dividing the disease into different subgroups according to the codon129 genotype and PrP^{Sc} conformation[23]. The *PRNP* genotype is homozygous or heterozygous for methionine (M) or valine (V) at codon 129 classifies cases into those with methionine homozygosity (MM), valine homozygosity (VV) or heterozygosity (MV). For example, the 'classical' sCJD phenotype of advanced age at onset, rapidly progressive dementia, early and prominent myoclonus and ataxia with a short duration of illness is seen in MM or MV individuals with PrP^{Sc} Type 1 (MM1 or MV1) whereas VV2 patients have a cerebellar variant, late dementia and longer duration of illness. In total, 6 clinical phenotypes have been described. The following tables summarise the key subtypes and their clinical and neuropathological characteristics. However, the mechanisms underlying the links between particular molecular and clinical findings are yet to be established. At neuropathology, spongiform change, astrocytosis and neuronal loss are observed, with amyloid plaques present in the minority. Typically, the cerebral cortex is involved, but the severity and distribution of neuropathological changes is variable although this is not discussed in detail here[23].

1.4.2.1.1 The Molecular Classification of Sporadic CJD (Adapted from Parchi et al)

Table 1 Disease duration and age for each molecular subtype

sCJD Subtype	Age at Onset (years)	Disease Duration (months)
MM1 or MV1	70.1 (48-86)	4 (1-24)
VV2	64.5 (45-83)	6.3 (3-18)
MV2 Kuru	65.4 (48-81)	15.8 (5-48)
MM/MV2 cortical	67.8 (61-75)	20 (12-36)
MM2 thalamic	52.3 (36-71)	15.5 (8-24)
VV1	39.3 (24-49)	15.3 (14-16)
MM/MV 1+2 cortical	68.6 (42-89)	4 (1-26)
MV2 kuru	NA	NA
VV2 +1	69.3 (59-85)	6.5 (3.5-13)

Table 2 Typical Clinical Features of sCJD Molecular Subtype

sCJD Subtype	Clinical Features
MM1 or MV1	Typically rapidly progressive dementia and myclonus Ataxia in 50% Visual impairment in 30%
VV2	Ataxic onset with dementia developing later
MV2 Kuru	Duration can exceed 2 years Dementia and ataxia
MM/MV2 cortical	Myoclonus, pyramidal signs and dementia Ataxia less common
MM2 thalamic	Frequently insomnia Psychomotor hyperactivity and motor problems Ataxia
VV1	Progressive dementia with pyramidal signs and myoclonus
MM/MV1+2 cortical	Similar as MM1 Phenotype depends on the predominance of either PrP^{Sc} type 1 or 2
MV2 Kuru +2 cortical	NA
VV2 + 1	Not dissimilar to VV2

1.4.2.2 Variant CJD (vCJD)

Ten years after the recognition of the initial case of BSE, the first case of an atypical form of CJD termed 'new variant' CJD in humans was identified igniting concerns that BSE had now been transmitted to humans via consumption of infected meat products[111, 112]. Following a series of experimental studies in mice, striking similarities were found particularly in regard to lesion profile and incubation periods [113,114.] The definitive association between BSE and vCJD resulted from the characterisation of primary and secondary transmission of 10 cases of vCJD mice[115]. Both CNS and peripheral tissue was transmitted and in all cases, the transmission characteristics including incubation period and abnormal prion protein deposition patterns were identical to BSE[115].

Since the first identified cases of vCJD in 1996, there have been 178 probable or definite cases identified in the UK. The annual number of deaths was maximal in 2000 with 28 deaths but after 2006, deaths from vCJD have declined significantly[116]. Worldwide, the majority of the cases have occurred in the UK. However, 51 cases have now been reported in other countries, with most of these occurring in France which is thought to be related to the level of importation of beef products between 1985 and 1995[117].

In comparison to sCJD, these patients have been much younger with a mean age of onset of 28 years old[118]. In addition, their presentation, disease duration and neuropathological findings are distinct from those seen in other human prion diseases[112]. The median survival post diagnosis is 14 months[118]. The heterogeneity of sCJD has been partly related to *PRNP* 129 genotype and PrP protein type. Variant CJD is more homogenous with the majority codon 129 MM[25]. Definite and probable cases of vCJD have been predominantly codon 129 MM genotype which is in contrast to the normal distribution (42% MM, 47% MV and 11% VV) suggesting an association between vCJD susceptibility and/or incubation period and genotype.

However, there has been one definite and one possible case that were both codon 129 MV[25, 112, 116].

Transmission studies using gene targeted mice expressing human PrP^{C} and carrying each of the codon 129 genotypes have suggested that there is potential for human to human transmission of vCJD and that this may be feasible in all genotypes[112, 114]. Further studies by Bishop et al demonstrated that the transmission efficiency varied in the order of MM>MV>VV with differing pathological characteristics for each genotype[112,119]. The greatest transmission efficiency occurred in mice expressing codon 129 MM and these mice demonstrated the earliest onset of the disease. In contrast, MV mice were shown to have longer incubation times[119]. This suggests that in humans, all genotypes have the potential to be affected with different genotypes manifesting disease in different ways[112]**.** Of concern is that MV and VV individuals may display long asymptomatic or subclinical incubation periods that may exceed normal lifespan or even remain permanently subclinical which obviously has public health implications.

In humans, PrP^{Sc} was discovered in the spleen and cervical lymph node of a *PRNP* 129 heterozygote asymptomatic individual who died of non-neurological disorder 5 years after receiving a blood transfusion[119]. The donor of the blood later went on to die from vCJD (vCJD and blood transfusion will be described in more detail under 'Iatrogenic CJD'). Transmission studies using the PrP^{Sc} from this individual's spleen were successful[119]. In addition, in 2009, a possible case of vCJD in a codon 129 heterozygote was not confirmed due to a lack of post mortem[120]. Retrospective studies of anonymised tonsil and appendix samples have demonstrated PrP^{Sc} in all 3 genotypes providing further support that all genotypes can be affected and add further concern to the possibility of subclinical infection and long incubation times[121, 122]. The concept of asymptomatic carriers of prion infectivity will be discussed further later in the chapter.

In addition to age at onset and duration illness, the clinical symptoms and signs are different in comparison to sCJD. Early in the illness, patients usually experience prominent psychiatric features or behavioural problems and painful sensory symptoms which often dominate for the initial 6 months. Psychiatric manifestations

including apathy, depression, anxiety are often the sole symptom for several months. Other neurological signs appear later and included cerebellar signs and gait ataxia, myoclonus, cognitive decline, involuntary movements followed by akinetic mutism in the end stages of the disease[25].

There are no completely reliable non-invasive tests to diagnose vCJD with the gold-standard continuing to be neuropathology. However, the presence of abnormal prion proteins in a variety of tissues other than the brain has been exploited by a number of studies with some promising results. Moda et al used a PMCA assay technique to try and amplify minute amounts of PrP^{Sc} in the urine of variant, sporadic and genetic forms of CJD as well as a host of controls which included both healthy individuals and those with a non-prion neurodegenerative disease. 13/14 confirmed cases of vCJD returned a positive test and none of the 224 non vCJD cases were positive. No cases of genetic CJD or sporadic CJD returned a positive result. This produced a sensitivity of 92.9% and specificity of 100% although it was acknowledged by the authors that a larger study was needed in order to provide statistical significance. Similar studies have been employed using blood[123]. Collinge et al developed a prototype assay that exploited the affinity PrP^{Sc} has for metal surfaces. The assay included a stainless steel powder combined with direct immunodetection. Results indicated a sensitivity of 70% and specificity of 100% although it was acknowledged that it remains unclear whether this sensitivity is high enough to detect abnormal prion protein in suspected asymptomatic carriers of the condition who are considered at risk. The levels of abnormal prion protein are thought to be even lower in these individuals than those who actually have the condition and so the assays reliability in this regard remains uncertain without a larger cohort study[124]. Soto et al examined the blood from a cohort of patients that included 14 confirmed vCJD cases and 153 non prion neurological cases. They employed a PMCA technique to amplify small amounts of PrP^{Sc} to detectable levels. The PMCA study had a sensitivity and specificity of 100%[125]. Bougard et al produced similar findings using a PMCA technique, including a positive result in two subclinical cases that later went on to develop vCJD[126]. Although promising as a non-invasive screening and diagnostic test, it has been recognised by authors that larger studies are required in this area.

Tonsillar biopsy can also be useful, reflecting the lymphoid involvement in vCJD, although this test is invasive and not without risk[122]. The EEG typically shows non-specifically slow-wave abnormalities or is normal[127]. MRI brain imaging characteristically shows symmetrical hyperintensity of the pulvinar nucleus of the thalamus, changes which in the correct clinical context are highly sensitive and specific for vCJD[118]. In addition, CSF 14-3-3 analysis is less useful in vCJD in comparison to sCJD being less sensitive with approximately 50% of cases returning a positive result[128]. Highly sensitive and specific diagnostic criteria, encompassing the core clinical features, results of MRI imaging and neuropathological features have been formulated and validated[118].

1.4.2.3 Iatrogenic CJD (iCJD)

Iatrogenic transmission of CJD has occurred in many countries across the world. However, cases of iatrogenic CJD have significantly declined with occasional cases with exceptionally long incubation periods emerging. The majority of these outbreaks were related to contaminated growth hormone and dura mater grafts derived from human cadavers with undiagnosed CJD infections[129]. A small number of cases were caused by neurosurgical instrument contamination, corneal grafts, gonadotrophic hormone and secondary infection with variant CJD transmitted by transfusion of blood products[130]. No new sources of disease have been identified. Although blood and blood product transmission has been reported with vCJD, the cases are really not classed with iCJD and the modes of transmission have not been reported with other forms of human prion disease (details are discussed in 1.4.2.4.1.3 below). Current practices focus on improved recognition of potentially infected persons until a blood or other simple screening test for the detection of preclinical infection is validated for human use.

The first case of iatrogenic CJD was reported in 1974 when a 55 year old patient received a corneal transplant from an infected cadaver and died 18 months post exposure[131]. Other sources of infection later emerged including stereotactic depth

electrodes, neurosurgical instruments, cadaveric dura mater and pituitary glands[132]. Some incubation periods have been exceptionally long (years, sometimes decades) which poses a significant challenge for public health teams whose introductory measures may help prevent transmission of further cases but are redundant when it comes to preventing cases of an already infected individual in the asymptomatic phase of the disease. The incubation periods in the worldwide cohort of iCJD cases are markedly variable. The shortest incubation periods occur on those exposed to neurosurgical instruments on the CNS (1-2.3 years). The longest incubation periods occur in human dura mater recipients (1.3-30 years) and cadaver derived growth hormone and gonadotrophin recipients (5-42 years). These prolonged incubation periods are not dissimilar of those occurring in Kuru where incubation periods of greater than 40 years have been reported[129, 132].

1.4.2.3.1.1 Human Growth Hormone

The current worldwide total of growth hormone associated cases of CJD is 226. Most cases occurred in France, the United Kingdom and the United States. The use of pituitary-derived hGH in the treatment of primary and secondary growth hormone deficiency in the United Kingdom began as a clinical trial in 1959 and became a centrally administered National Health Service (NHS) activity in 1976[132]. The first case of iCJD secondary to hGH derived from cadaveric pituitary glands was reported in 1985 in the United States of America and in the UK[133, 134]. The mean incubation period in the United Kingdom was 20 years[135]. However, predicting incubation periods in cases of human pituitary hormone related CJD is difficult as patients are frequently treated over a period of years[132]. The use of cadaveric growth hormone to treat growth hormone deficiency was widespread from the late 1950s to the 1980s, with little or no screening of donors[132].

1.4.2.3.1.2 Dura Mater

The worldwide total of dura mater associated cases of CJD is 228 and new cases continue to emerge. However, in contrast to hGH cases, only 8 recipients of hDM have developed iCJD in the UK with incubation periods ranging from 3.8-14.8 years[132, 136]. The majority of cases were reported in Japan where the use of such grafts during neurosurgery was particularly common. The most recent cases occurred in Austria, South Korea and the Netherlands in 2011. Incubation periods ranged between 1.3 to 30 years[137]

1.4.2.3.1.3 Blood

The UK has the highest incidence of vCJD in the world as described[112]. During the initial stages of the vCJD epidemic, transmission via blood transfusion from infected individuals was considered a minimal risk[112]. Despite this however, significant efforts were made to trace the fate of all blood components used for transfusion from donors known to have CJD[12]. In addition, a number of transmission studies were conducted to assess the ability of prion infection to transfer via blood. Sheep studies initially demonstrated that blood components collected either in the subclinical or clinical phase of the disease contained titres of infectivity that were sufficient for transmission to recipients after a single blood transfusion. In addition, the number of recipients that developed disease supported that blood was a very efficient means of transmission[12, 112]. From 2004, 4 cases of blood transfusion related vCJD infection have been documented in the UK[11, 138.] The transfusions occurred between 1996 and 1999. All donors of the blood components were asymptomatic at the time of donation although it was later confirmed that they all died from vCJD. Of the 4 blood component recipients, 3 developed clinical signs supportive of vCJD and this was later confirmed on post mortem examination. These cases were all codon 129 MM in the *PRNP* gene[138]. Interestingly, the remaining case was heterozygous at codon 129 and did not display any signs of a neurological disorder including vCJD.

There was also no evidence of vCJD in the individual's brain and the patient died of an unrelated condition[138, 139]. However, disease associated prion protein was identified in the spleen and cervical lymph node as described earlier in chapter.

1.4.2.3.1.3.1 Current Prevention Strategies

The optimal method of abolishing secondary iatrogenic infections is to prevent primary infections. In the mean-time, without a test to identify persons who are in the asymptomatic phase of the disease, it is impossible to entirely eliminate the risk between human to human tissue transfer which is of significant public health concern. At present we are obliged to rely on default strategies which include: identification and donor deferral of persons considered at risk, inclusion of prion reduction steps in the sterilisation of penetrating instruments and the processing of therapeutic tissues and fluids[132].

1.4.2.3.1.3.2 Risks Posed by Asymptomatic vCJD Infection

The possibility of iatrogenic infection resulting from the transfer of tissues and bodily fluids remains real despite the apparent decline in the epidemic of BSE and vCJD. To date, vCJD has occurred in MM and MV *PRNP* genotypes although is more common in homozygous individuals. Data already discussed has demonstrated that MV cases can be associated with a longer incubation time which raises concern about the possibility of subclinical or asymptomatic infectivity and thus potential for iatrogenic transmission. This has prompted a series of studies aiming to establish the potential prevalence of asymptomatic individuals that are possibly in the subclinical phase of the condition and may be infectious. Variant CJD differs from other human prion diseases due to the widespread involvement of lymphoid tissues. A small number of cases later confirmed to have had vCJD and previously had their appendices removed before the onset of clinical symptoms revealed that the disease associated prion protein was detectable up to 2 years prior to the onset of clinical

disease[139, 140]. This finding prompted a larger retrospective study of tonsils and appendices from laboratories throughout the UK in order to provide an estimate of asymptomatic carriers of the condition. A series of anonymous studies were carried out and in 2004, the first estimated prevalence of asymptomatic cases was reported as 237 per million. Further studies have demonstrated a prevalence of 493 per million[**1**21, 140]. These findings identify a number of important implications and support the need for ongoing surveillance, particularly with regard to the risk of secondary transmission from asymptomatic subclinical cases via surgical instruments or blood transfusion.

1.4.2.4 Variable Protease sensitive Prionopathy (VPSPr)

A relatively recently identified, and presumably sporadic, human prion disease called Variable protease sensitive prionopathy or VPSPr was initially described in 2008 with 11 cases reported by that national prion surveillance unit in Cleveland Ohio. A further two cases were reported with all initially codon 29 VV genotype[141]. In 2010, a further 15 cases were identified which included both MV and MM genotypes[142]. This novel type of human prion disease harbours a distinct neuropathological and biochemical profile that differs from CJD although has some similarities to GSS[143]. The lack of risk factors for iatrogenic CJD and *PRNP* mutation support a sporadic form of prion disease and it has been suggested that this could be the sporadic form of GSS[143, 144]. In comparison to sporadic CJD, the clinical differences include a slower disease progression and presentation with some of the more atypical forms of dementia such as Frontotemporal lobe dementia, Lewy body disease and normal pressure hydrocephalus[145]. Biochemical differences include the fact that the abnormal form of the prion protein is much less resistant to protein digestion. In addition, the neuropathological features are unusual and include microplaques within the cerebellum and thalamus[142, 144].

Human to human transmission of prion diseases is of significant concern, particularly in relation to public health. Studies on transgenic mice have shown that sporadic CJD is readily transmitted in the presence of human PrP with the development of the recognisable clinical signs and the typical neuropathological hallmarks of the condition[146]. Studies have demonstrated the transmissibility of VPSPr is much less efficient than sCJD[147, 148] but not entirely negligible[144]. These findings support the need for ongoing surveillance to fully establish the expanding spectrum of human prion disease.

1.5 Inherited CJD

Inherited CJD is historically classified into three forms according to their clinicopathological features. These include genetic CJD (gCJD), Gerstmann Sträussler Scheinker syndrome (GSS) and familial fatal insomnia (FFI). Inherited CJD is an autosomal dominant condition related to underlying mutations of the prion protein gene (*PRNP*). Genetic analysis of the sCJD and vCJD cohort is important as genetic prion disease cannot always be distinguished from clinically from other forms of prion disease and a family history is absent in approximately 50%. For example genetic CJD can also present with a rapidly progressive dementia and motor syndrome with similar neuropathological features[21].

1.5.1 Genetic CJD

Genetic prion diseases were initially recognised in at the turn of the 20th century[21]. Genetic CJD (gCJD) presents in a similar fashion to sCJD with a rapidly onset dementia with death occurring within a few months of symptom onset. This supports the importance of genetic testing in all cases of sCJD as gCJD has the potential to masquerade as sCJD[21, 149]. The first mutation discovered to cause prion disease was the 6- octapeptide repeat insertion (6-OPRI)[150,151]. The identification of several other point and insertional mutations were identified shortly after this and new mutations are still be discovered. Over 20 mutations have been identified in the opening reading frame (ORF) that have been linked to genetic disease phenotypes, the commonest being E200K which accounts for over 70% of families worldwide[21, 149].

The clinicopathological phenotype can vary significantly, with differences reported in families with the same mutation. As with sCJD, the influence of *PRNP* codon 129 polymorphisms has a major influence on upon the specific manifestations of the disease[21, 18].

1.5.2 Gerstmann Sträussler Scheinker Syndrome (GSS)

GSS is an autosomal dominant form of inherited prion disease with several point mutations in the *PRNP* gene[152]. In 1936, J. Gerstmann, E. Sträussler, and I. Scheinker reported an Austrian kindred that presented with slowly progressive cerebellar ataxia and late dementia, with an autosomal dominant pattern of inheritance, which is now considered the first known GSS family (*PRNP* P102L mutation identified later)[153]. The clinical phenotype shows marked heterogeneity, even amongst family members with the same mutation which can make the diagnosis challenging and sometimes missed[21, 153]. The commonest mutation is the P102L mutation cases typically presenting with a slowly progressive cerebellar ataxia and late onset dementia. There are often pronounced cognitive and psychiatric features and some clinical presentations are not dissimilar to sCJD[154]. The age of onset of GSS is relatively early but the disease progresses slowly, with an average disease duration of 3-10 years although this can be shorter[21]. A GSS case attributable to A133V mutation resulted in a rare clinical phenotype similar to that of progressive supranuclear palsy. Several other pathological mutations that result in GSS have also been described[21].

1.5.3 Fatal Familial Insomnia (FFI)

FFI is a subtype of genetic CJD. They are both linked to the same mutation of codon 178 of the prion protein gene but remain two clinically and pathologically distinct diseases[21]. FFI is the third most common inherited prion disease and the most common inherited prion disease in Germany[152]. The term FFI was first used by Lugaresi et al. in 1986, in the description of an Italian family with progressive insomnia, dysautonomia, motor signs, and thalamic degeneration[155]. The neuropathological findings suggested FFI was a prion disease, but confirmation of it being genetic did not occur until 1992, when a PRNP D178N-codon 129M mutation was identified in the kindred[156]. Since this time >100 FFI pedigrees have been identified[21]. The genetic difference between FFI and genetic CJD lies in a polymorphism at codon 129. FFI occurs when an individual has a D178N mutation and methionine at codon 129 of the mutated allele. If the mutated allele carries valine then the disease phenotype resembles genetic CJD[157]. Usually the diagnosis is made around the 5th decade although a wide range age of onset has been reported[21, 158]. The core features are related to thalamic dysfunction and include profound disturbance of the sleep-wake cycle and dysautonomia[159]. However, clinical variability in the clinical phenotype is well recognised amongst mutation carriers[160]. Interestingly, cases of sporadic fatal insomnia (SFI) have a clinical phenotype that is indistinguishable from its genetic counterpart but there is no detectable PRNP mutation. This form is considered part of the sCJD spectrum[159].

1.5.4 Codon 129

The *PRNP* codon 129 M/V polymorphism has potential effects on susceptibility to prion diseases, the incubation period in infectious forms and, as has been discussed above, the clinic-pathological manifestations of the disease[23]. The polymorphic residue at codon 129 of the prion protein gene (methionine/valine) has been studied extensively[23]. There is variation in genotype frequencies according to geographical region with a pattern of increasing homozygosity extending from Western to Eastern countries[161-163]. In the majority of countries studied, MM and MV frequencies are approximately 40-50% [161-163]. A study in Poland reported 45% MM, 39% MV and 16% VV[164]. However, a study in a Turkish population demonstrated a slight increase in homozygosity with 57% of the normal population being MM, 34% MV and 9%VV[165]. In Korea, it was even higher with MM 94%, MV 5%, VV 0.19%[166]. Studies in Japan have also demonstrated a large difference where the normal population frequencies are MM 92%, MV 8% and VV 0%[167].

Codon 129 homozygosity is considered a risk factor for the development of human prion disease[162, 168]. 70% of sporadic CJD and the majority of variant CJD cases are MM as previously discussed[169]. Homozygosity has also been shown to be a risk factor and reduce the incubation time for human growth hormone associated CJD, iatrogenic CJD and Kuru[170, 171]. A French/UK study found an excess of VV CJD in iatrogenic CJD compared to sCJD[170]. A further study of dura mater graft associated CJD in Japan divided cases into 'typical' and 'atypical' cases. All cases were classified as MM apart from one case of atypical dura mater CJD which was documented as MV. This raised the possibility of different strains[137].

In addition, analysis of the prion protein gene and codon 129 has produced some important findings as discussed earlier in the chapter. Given that the majority of clinically probable and neuropathologically confirmed cases of variant CJD have been MM genotype provides good evidence that this genotype is classified as a risk factor. With regard to the 4 cases of vCJD related to blood transfusion (discussed earlier in the chapter), 3 of these cases were MM and 1 was found to be MV. However, in the MV case, there was no clinical or neuropathological evidence of

vCJD in the recipient but disease related abnormal prion protein was found in the spleen and one lymph node[11]. It is suspected that the recipient was therefore in the preclinical phase of the condition and therefore impossible to know whether they would have proceeded to the clinical phase of CJD if they had lived longer[11, 162]. In addition, 2 or 3 samples in an anonymous appendix study in the UK, were positive for deposition of prion disease associated with PrP^{Sc} were genotyped as VV suggesting that these polymorphisms are potentially associated with longer incubation periods and opens up the possibility that a second wave of vCJD may occur in the future[162, 172].

Currently, the diagnosis of sCJD is based on the clinical presentation in addition to conventional diagnostic tests that include EEG, MRI and CSF 14-3-3[173]. The influence of polymorphisms at codon 129 on the results of these tests is well recognised with growing concern that these tests may miss uncommon variants of the condition[174]. One study analysed the value of these commonly used clinical tests for the clinical diagnosis of all 6 subtypes of sCJD. Results demonstrated that EEG was reliable for MM1 and MV1 subtypes only. 14-3-3 held a high sensitivity for all subgroups but not MV2. However, MRI was especially reliable for MV2 and overall had a sensitivity of 70% irrespective of subgroup[174]. These studies were further supported by a study in Catalonia[175]. In 2006, a further paper found EEG to be a useful test in the more classic presentations of sCJD (i.e. MM and MV but not VV)[176]. A further study included a cohort of 2451 definite cases of sCJD of which 743 underwent molecular subtyping. Results found that illness duration significantly influenced the result of 14-3-3, however, MRI was not influenced by this and had an increased sensitivity to VV2 cases[177]. EEG was also influenced by age at onset and illness duration and was reliable in MM1 subtypes but less so in MV1, MV2 and VV2[177]. Overall, 14-3-3 was the most reliable test of all 3 but less likely to be positive in the less common subtypes such as MV2 and MM2[177]. However, a further paper contradicts this finding and reports that in fact 14-3-3 is very useful in identifying cases of MV2 and MM2[178]. A further paper has shown that the MRI lesions vary across different subtypes. Pathologically confirmed sCJD cases with codon 129 genotype (MM, MV, and VV), PrP(Sc) type, and fluid-attenuated inversion recovery (FLAIR) or diffusion-weighted imaging (DWI) were collected in

seven countries. MRI scans were evaluated in 211 CJD patients (98 MM1, 23 MM2, 19 MV1, 30 MV2, 9 VV1, and 32 VV2). Basal ganglia hyperintensities occurred most frequently in MV2, VV2, and MM1 subtypes (79, 77, and 70%). Wide cerebral cortical signal increase was most common in VV1, MM2, and MV1 subtypes (86, 77, and 77%). Thalamic hyperintensities occurred most often in VV2 (45%) and MV2 (43%). The most consistent finding across most subtypes was high signal in basal ganglia, with these abnormalities found in 63% (FLAIR) and 71% (DWI) .[179]

1.5.5 PrP^{Sc} Glycotype

In addition to the polymorphism at codon 129, the specific glycotype of PrP^{Sc} that accumulates in the brain also bears relevance to the clinic-pathological characteristics of prion disease. Two major glycotypes have been validated for sCJD, Type 1 and Type 2 [23, 180]. The glycotypes are mainly distinguished by the electrophoretic migration of the unglycosylated fragments. The unglycosylated PrP^{Sc} Type 1 fragment migrates at 19 kilodaltons whereas Type 2 migrates at 21 kilodaltons. These PrP^{Sc} types are presumed to reflect the differences in the susceptibility of distinct PrP^{Sc} strains to protease digestion due to accessibility to different cleavage sites[180]. Codon 129 genotype and PrP^{Sc} glycotype have been shown to be associated with the clinical heterogeneity of sporadic CJD with 6 classifications reported by Parchi et al as previously described in the chapter. Overall, codon 129 genotype, age at onset, glycotype and sex act as prognostic indicators for sporadic CJD[110].

1.6 Classification and Diagnosis of Sporadic CJD

Currently, there is not a disease specific, non-invasive test available for the diagnosis of sporadic CJD. Post mortem examination of brain tissue demonstrating the presence of the abnormal prion protein PrP^{Sc} remains the definitive means of diagnosis[173]. Brain biopsy is a feasible option although carries risk for the patient and is infrequently performed[181]. Atypical presentations can prove a diagnostic challenge and there can be a delay in diagnosis which can be distressing for family members. In addition, identifying potentially treatable alternative diseases and also preventing secondary transmission are both important.

In 1979, Masters et al developed diagnostic criteria to aid the diagnosis of CJD[182]. These have been modified over the years, most recently in 2009[173]. The current diagnostic criteria rely on the clinical presentation in association with investigations including electroencephalogram (EEG), MRI brain and 14-3-3 protein in the cerebrospinal fluid (CSF)[173]. Although these tests are useful, they do not provide the necessary degree of sensitivity and are not specific to prion disease and therefore lack diagnostic accuracy[173, 176, 177, 178, 179, 183, 184, 185]. In addition, strict compliance of the criteria has the potential to result in failure to recognise cases, particularly in the early phases of the disease when non-specific features predominate and the traditional signs have not yet developed[186, 187]. The differential diagnosis of rapidly progressive dementia is broad and it is important to confirm or exclude CJD early so that potentially treatable conditions that may present in similar ways to sporadic CJD are not missed. In addition, a wide range of focal signs at onset have been reported in patients with sporadic CJD that may suggest alternative diagnoses[186]. Examples of focal signs include cerebellar ataxia[187], visual symptoms[188], aphasia[189], alexia without agraphia[190] and alien limb phenomenon[191]. Motor weakness such as hemiparesis, monoparesis and focal seizure activity may also occur[192, 193]. In many of these cases, the presenting focal manifestation can be of acute onset, often leading to an incorrect initial diagnosis of stroke[194]. More global presentations may suggest autoimmune encephalitis, paraneoplastic disorders or toxic and metabolic

disorders[186]. A diagnosis of sporadic CJD requires the exclusion of other potentially treatable disorders.

Supportive investigations such as EEG, MRI and 14-3-3 have relatively low general diagnostic sensitivity and specificity although they may be very useful in specific clinical contexts[173, 176, 177, 178, 179, 183, 184, 185]. The inclusion of certain MRI brain imaging sequences including fluid-attenuated inversion recovery (FLAIR) and diffusion weighted imaging (DWI) has improved the diagnostic yield and helped differentiate from other causes of rapidly progressive dementia although even these are not absolutely specific for sCJD[179, 184]. The characteristic findings on EEG in sCJD are periodic sharp wave complexes (PSWCs) although these are non-specific: they can be present in a wide variety of other conditions[195]. In the early stages of sCJD, it is not uncommon for the EEG to demonstrate non-specific slowing, lateralised abnormalities such as periodic lateralised epileptic discharges (PLEDs) or focal abnormalities[176,186, 195, 196]. It can take days or weeks for PSWCs to manifest, if at all[186]. Elevated CSF 14-3-3 levels, while have a reasonable sensitivity for sCJD, have a relatively low specificity (for example, it can be positive if the patient is suffering from seizures or indeed any neurological condition that can lead to acute neuronal damage)[185, 197].

Unfortunately, regardless of the criteria, sCJD with early focal features or atypical manifestations and sCJD in the early phases of the illness with no characteristic EEG or MRI changes may escape an early diagnosis[186]. This may result in repeated or unnecessary investigations and a subsequent delay in diagnosis. This of great importance given the public health implications of this, potentially transmissible and incurable disease. There is thus a need for a relatively simple, disease specific, reliable diagnostic test that can provide an earlier and more accurate diagnosis.

The unique feature of prion disease is the presence of the abnormal prion protein and its ability to replicate and aggregate. This seeded conversion of PrP^{C} to PrP^{Sc} has been exploited by a variety of assay techniques to detect the abnormal prion protein with sufficient sensitivity to enable the detection of PrP^{Sc} in biological tissues such as CSF, blood, urine and olfactory mucosa in patients with prion disease[198-206].

Protein amplification techniques have revolutionised the diagnosis of prion diseases and provide a platform for accurate antemortem diagnosis for prion diseases. They exploit the ability of PrP^{Sc} to induce conformational change in PrP in a continuous fashion. This allows the small amount of PrP^{Sc} found in biological fluids such as blood and CSF to be amplified to a threshold where conventional laboratory techniques can detect it. The most widely used protein amplification techniques include Protein Misfolding Clyclic Amplification Assay (PMCA) with western blotting detection or alternatively surround optical fibre immunoassay (SOFIA)[198, 199, 200, 201] and Real Time Quaking Induced Conversion (RT-QuIC) which uses thioflavin-T immunofluorescence to detect PrP^{Sc} or western blotting[202, 203].

1.6.1 Protein mis-folding amplification assay (PMCA)

PMCA assays exploit the fundamental replication mechanism of prions as detailed above. Test samples are combined with homogenates of brain tissue or cells that contain PrP^{C} which act as the source of PrP^{C}. Disease associated PrP^{Sc} found in brain and peripheral tissues (blood, urine, spleen, saliva) is added, thus inducing a seeding reaction and amplifying the level of PrP^{Sc} [198-201, 204, 205, 206]. To optimise the sensitivity of PMCA, the mixture undergoes a series of sonication and rest cycles which breaks up the aggregated or seeded PrP^{Sc} into several smaller fragments that will also start to seed and amplify further leading to more aggregation. In addition, sensitivity is further improved by adding small amounts of the reaction products to fresh sources of substrate[207].

However, there are limitations that have been identified with this technique. The use of multiple cycles of sonication can induce spontaneous aggregation of substrate in the absence of any seed[208]. In addition, the requirement of multiple sonication cycles to maintain adequate sensitivity is time consuming and labour intensive[205]. The need for brain material as a substrate also has limitations. For example, having a consistent and continuous substrate supply can be difficult [209, 210]. The lack of

consistency between laboratories despite using similar protocols has also been an issue[109].

1.6.2 Real Time Quaking Induced Conversion

To address these practical short-comings, a further assay was introduced and developed based on the PrP^{Sc} seeding process called Real Time Quaking Induced Conversion (RT-QuIC)[202, 203]. The RT-QuIC test has been adapted since its initial development and now has the ability to detect many types of prion seeding activity in multiple host species and specimen types including those of particular diagnostic significance such as CSF, blood, saliva and nasal brushings[202, 203, 2011-219] . This test has shown considerable promise as a highly specific diagnostic test for the diagnosis of sporadic CJD[211-219].

In the RT-QuIC assay, recombinant prion protein is used as a substrate ($rPrP^{Sen}$) and is mixed, or seeded, with a small amount of PrP^{Sc} from CSF or brain tissue and is set up in a 96 well microtitre plate thus facilitating a high throughput assay. The reaction mixture leads to PrP aggregate formation and these are broken up by using intermittent shaking rather than sonication cycles as employed by PMCA. Another superior feature of RT-QuIC is the 'built-in' detection facility. The reaction mixture contains Thioflavin-T (ThT), a dye that binds to amyloid aggregates. As the aggregates form, the kinetics of fibril formation can be monitored in real-time using standard fluorescent spectrophetometers ThT fluorescence is measured in relative fluorescence units (rfu) with saturation occurring at 65 000. After an initial 'lag phase' of 5-10 hours with seeded brain tissue and 30-40 hours with CSF tissue, there is a rapid increase in the fluorescent signal until it reaches a maximum at 40 hours for brain tissue and 90 hours for CSF[211]. A positive response was considered to have occurred if the average of the two highest readings at 90 hours was 10 000 rfu or greater. A cut off value of 10 000 rfu was chosen based on the mean of the first 20

non-CJD CSF samples analysed. A more detailed description of RT-QuIC analysis is given in the Methodology section[211].

Applications of RT-QuIC to panels of CSF from patients with and without the condition have shown sensitivities for the detection of sporadic CJD of 80-90% and specificities of 99-100%. These studies have indicated that RT-QuIC can achieve greater diagnostic performance than other CSF biomarkers for sporadic CJD[203, 211, 216]. Current alternatives for the definitive diagnosis of sporadic CJD involve invasive techniques such as brain biopsy for neuropathological confirmation of the disease in living patients[181]. As a much less invasive test with a high degree of diagnostic accuracy, RT-QuIC is now being implemented as a key diagnostic test.

1.6.3 Sporadic CJD Diagnostic Pathways

Within the United Kingdom, there is a national referral system for suspected cases of CJD in which clinicians notify the National CJD Research and Surveillance Unit (NCJDRSU) of patients who may or may not have the condition but in whom it is being considered a possibility. Cases are discussed in detail and initial investigations advised according to the EuroCJD diagnostic criteria, as previously described earlier in this chapter. Further description of the role of the NCJDRSU is provided in the Methodology section.

1.7 Rationale for Current Study

An early and accurate diagnosis of sCJD is important due to the aforementioned public health implications, to exclude CJD as a potential diagnosis early so that treatable neurological conditions are not missed and to ease distress of family members. Provisional studies have shown that RT-QuIC has the potential to identify cases of sCJD that conventional techniques such as 14-3-3 may miss and thus contribute to an early and more accurate diagnosis. At the time of writing, there had not been a prospective analysis of how useful RT-QuIC is in routine clinical practice and whether it has any added benefit over current antemortem diagnostic methods.

The aim of this study is to prospectively assess the diagnostic utility of RT-QuIC in routine clinical practice and determine the value in RT-QuIC in cases where there is diagnostic uncertainty using current diagnostic techniques and establish whether it can provide an earlier diagnosis. In addition, I will assess if certain clinical factors affect the RT-QuIC result, including codon 129 genotype, age at onset, symptoms at onset and duration of disease. Throughout the course of the study, I will also review the operational parameters (for example CSF volume, timing of lumbar puncture) of RT-QuIC and contribute to the optimisation and development of this new diagnostic test.

Chapter 2

Methodology

2 Methodology

2.1 The National CJD Research and Surveillance Unit (NCJDRSU)

'To monitor the characteristics of all forms of CJD, to identify trends in incidence rates, to study risk factors for the development of the disease and to contribute to improving the quality of care for those with CJD[116]

The National CJD Research and Surveillance Unit (NCJDRSU) was established in 1990 and has two principle functions which include surveillance of prion disease within the United Kingdom and research into the condition. It is comprised of a team of specialists which include clinical neurologists, neuropathologists and a variety of scientists. The NCJDRSU relies on the referral of clinically suspected cases by clinicians from throughout the United Kingdom. Notification is predominantly from neurologists, although we also receive referrals from other specialists including geriatricians and pathologists. As part of surveillance, the unit has a number of other roles including analysis of CSF for 14-3-3 protein, genetic analysis, molecular biological studies, review of MRI scans and neuro-pathological examination of tissue received from clinically suspected cases. There is a national referral system for suspected cases of CJD in which clinicians notify the NCJDRSU of patients who may or may not have the condition but in whom it is being considered a possibility. In addition, clinicians send CSF fluid to the 14-3-3 laboratory located within the NCJDRSU for analysis. These samples are from clinically suspected cases as discussed above, but also from patients who are being investigated by local physicians although not yet regarded as a suspected case for formal referral.

2.2 Assessment of Cases

The data period for this study was January 2012 to January 2013. During this time, I was the clinical registrar at the NCJDRSU. I played a pivotal role in the assessment and review of all referrals to the NCJDRSU. In addition, I was responsible for overseeing all requests for 14-3-3 analysis, reviewing all 14-3-3 results and re-assessing each individual case by discussing with the referring clinician to establish whether a formal referral is warranted.

All cases referred to the NCJDRSU that were being considered potential cases of CJD were offered a visit by the clinical registrar. Permission was sought from the patient's family and local consultant for an assessment to take place. During the visit, the patient (where possible) was interviewed and examined. A semi-standardised questionnaire was used to interview the family to obtain detailed clinical and epidemiological data. This included an accurate history of the illness, past medical history and previous operations relevant family history and occupational history. The results of relevant investigative findings were also recorded. Copies of medical records, neuroimaging and EEG were obtained following written consent from the next of kin. If the patient was referred after their death, then the family were contacted (with the permission from the local clinician or the general practitioner) and visited to complete the questionnaire, obtain the clinical history and obtain consent to review the medical records and relevant investigations. GP notes were also obtained after the patient had died.

2.3 Clinical Presentation

Data about the age at onset, date of symptom onset, clinical symptoms and signs at presentation and throughout the course of the illness were extracted from the available information from the questionnaire and medical notes. The onset was taken as the earliest date when symptoms that were deemed likely to be related to prion disease were noted by the patient's family, local clinician or general practitioner. Where the exact date of symptom onset could not be provided (e.g. the month was given), then the 15th day of the month was used. Therefore, a certain amount of error will be present regarding the exact date of onset. The disease duration was calculated using the date of onset and the date of death inclusive. The presenting symptoms were extracted from the questionnaire. The mode of symptom onset was determined using EuroCJD definitions (Mrs Terry Lindsey, personal communication, www.cjd.ac.uk)[116].

The presence or absence of clinical symptoms and signs as stipulated by the EuroCJD Diagnostic Criteria 2009 were recorded[173].

2.4 Investigations

The investigations conducted for each case were chosen as clinically indicated by the referring clinician. Some cases were discussed with clinical team at the NCJDRSU in advance and advice provided regarding the most appropriate tests. Not all patients underwent CSF examination, EEG and neuroimaging, either because the patient was too unsettled for such investigations or because they were not felt to be clinically indicated.

2.4.1 CSF Analysis

The CSF laboratory within the NCJDRSU provides a national and international diagnostic service for the analysis of CSF 14-3-3 and other brain specific proteins. Since 2012, the laboratory has helped establish the RT-QuIC technique in many European countries. The NCJDRSU receives approximately 300 CSF samples for 14-3-3 analysis per annum.

For the purposes of this study, all CSF samples accepted for 14-3-3 analysis between January 2012 and January 2013 were also tested using RT-QuIC. RT-QuIC analysis was conducted by Dr Lynne McGuire and Dr Alison Green, post doctorate research scientists. Both of these tests were performed according to previously established methodology[203]. Few samples of CSF 14-3-3 were conducted in the National Hospital for Neurology and Neurosurgery in London.

During the course of this study, the RT-QuIC assay has been adapted and improved with the development of, a so called, ‘second generation’ RT-QuIC. For the purposes of this study, the ‘first generation’ RT-QuIC assay was employed. The differences between the two assays are discussed in Chapter 4.

The first generation RT-QuIC assay employed the standard reaction mixture of recombinant substrate, seed and buffer. In this study, the buffer composition included a10mM phosphate buffer (pH 7.4), 170mM NaCl (total 300mM including

phosphate buffer), 10µM Thioflavin-T (ThT) and 10µM ethylenediaminetetracetic acid tetrasodium salt (EDTA). This was mixed with the recombinant substrate which in this study was 0.1mg/ml of full length hamster PrP (23-231). Reactions were prepared in a black 96 well, optical bottom plate in volumes of 98µl for brain homogenate (BH) seeded reactions and 85µl for CSF seeded reactions. 2µl of diluted brain homogenate or 15µl of diluted CSF were added to the wells for a final reaction volume of 100µl. Each sample was run in quadruplicate, allowing 4 control samples (unseeded, non-neurological brain homogenate, Alzheimer's disease brain homogenate, sCJD MM1 brain homogenate) and 20 CSF samples to be tested on one plate. The plates were sealed and incubated in a BMG OPTIMA FLUOstar plate reader at 42ºC for 90-120 hours with intermittent shaking cycles. ThT fluorescence measurements were taken every 15 minutes.

The plate reader measures ThT fluorescence in relative fluorescence units (rfu) with saturation at 65 000. The average fluorescence for each quadruplicate sample was measured against time. Following a lag phase of 5-10 hours for sCJD BH and 40 hours for sCJD CSF, a positive sample displayed a rise in rfu. A positive response was considered if the average of the two highest readings at 90 hours was 10 000 rfu or greater. A cut off value of 10 000 rfu was chosen based on the mean of the first 20 non-CJD CSF samples analysed[211].

2.4.2 EEG Analysis

If an EEG was performed, copies of the representative pages were obtained either during the visit or after. Each EEG was independently reviewed by either Professor Richard Knight or Professor Robert Will. These were classified as either showing typical changes consistent with sporadic CJD or not typical changes. In the cases where a copy was not obtained and therefore not reviewed at the NCJDRSU, the local report was used.

2.4.3 Neuroimaging

If a MRI brain was performed, hard copies of the images were obtained either at the time of the visit or following assessment. The timing of the MRI scan in relation to the onset of the symptoms was also documented. The images were reviewed by an experienced consultant neuro-radiologist, Dr David Summers. Details of any abnormalities were documented with particular reference made to the presence of the typical findings accepted by the EuroCJD diagnostic criteria for sporadic CJD[165]. These included basal ganglia high signal +/- cortical high signal in three or more brain regions on Fluid Attenuated Inverse Recovery (FLAIR) or Diffusion Weighted (DWI) Sequences. In the instances where a hard copy of the MRI scan was not received, the report from the local radiologist was used.

Cases that did not meet the EuroCJD neuroimaging criteria, but demonstrated other recognised features of sporadic CJD such as multiple areas of isolated cortical ribboning were examined in detail. If the case was strongly suggestive of sporadic CJD clinically and the imaging showed cortical ribboning, with all other potential diagnoses excluded, then the MRI brain was considered a positive scan. This was based on the expert and experienced opinions of both the clinical team at the NCJDRSU and neuro-radiologist, Dr David Summers. However, for the benefit of classification, the EuroCJD criteria was strictly adhered to.

2.4.4 Genotyping

Provided the family of the patients who have been assessed have provided written consent, *PRNP* sequencing and codon 129 analysis was performed according to previously published methodology. Data was also obtained from genetic testing performed by the National Prion Clinic, London. Either blood or frozen brain tissue was used according to what was available.

2.4.5 Neuropathology

In cases that were reviewed and underwent a post-mortem, neuropathological brain tissue was examined at the NCJDRSU following provision of written consent from a relative. In some cases, tissue was not available and the post mortem was conducted at the National Prion Clinic in London, in which instance, a copy of the written post mortem report was obtained.

During the study period, immune-histological examination and prion protein typing was conducted by Dr Mark Head's team. Prion protein typing was carried out using Western Blot Analysis to determine the size and abundance of the different PrP^{Sc} glycoforms. Protein typing was classified according to their molecular weight into either Type 1 (non-glycosylated form with a molecular weight of 19kDa) or Type 2 (non-glycosylated with a weight of 21 kDa). If the di-glycosylated band predominates in Type 2 then it is termed Type 2B. If the di-glycosylated band does not predominate then it is classified as Type2A[218, 219].

During the study period, neuropathological analysis was performed by Professor James Ironside[220].

2.5 Case Assessment and Data Extraction

Between July 2011 and March 2014, I was the NCJDRSU clinical registrar. The study period extended between January 2012 and January 2013 and was a prospective study throughout this time period. I played a pivotal role in the assessment and review of all referrals to the NCJDRSU. In addition, I was responsible for overseeing all requests for 14-3-3 analysis, reviewing all 14-3-3 results and reassessing each individual case by discussing with the referring clinician to establish whether a formal referral was warranted. For the purposes of this study, all CSF samples accepted for 14-3-3 analysis during the study period were also tested

for RT-QuIC. Relevant clinical and investigation information was noted at referral and the patient was classified according to the current EuroCJD diagnostic criteria for sporadic CJD at that time. All formal referrals were offered a visit and the family interviewed (and the patient, where possible) using a semi-standardised questionnaire, obtaining detailed clinical and epidemiological data. Neurological examination of the patient was also carried out. As previously detailed, hospital and GP notes in addition to relevant investigations were requested and reviewed. Following the visit and review of the relevant investigations, each case was then re-classified according the current diagnostic criteria.

All cases were followed up by contacting the referring clinician. This included cases that did not meet criteria at initial referral. If the 14-3-3 or RT-QuIC were unexpectedly positive in this group, these cases were examined in further detail and a visit was offered if it was considered necessary. I created an Excel database and recorded the following data.

Table 3 Data Collection

ID Number	Location	Growth Hormone Case	Family Interviewed after death
Age at Onset	Date of Symptom Onset	Genetic Case	Date of Visit
Date of Birth	Disease Duration	Formal Referral	
Sex	CSF Referral	Date of Formal Referral	
CSF ID Number	Date of CSF Referral	Reviewed During Life	

I also recorded the mode of onset for each case using the following EuroCJD definitions (Mrs Terri Lindsay, NCJDRSU, personal communication) as illustrated in Table 3[116]. Where 'Other' was the designated mode of onset, details were recorded in free hand in the Excel database.

Table 4 Definition of Modes of Onset

Mode of Onset	Definition
Rapidly Progressive Dementia	The precise presenting symptom will vary from case to case, but the picture is an encephalopathic illness with dementia (and other neurological features), progressing rapidly over weeks to a few months, with no individual cognitive or physical deficit being present alone for more than two weeks.
Slowly Progressive Dementia	These cases present with a slowly progressive dementia, developing over months to years, without any other significant neurological features for the first six months.
Cortical Blindness (Heidenhain)	These cases present with impairment of visual acuity and/or field, progressing onto cortical blindness, without other significant clinical deficit for the first two weeks of illness. The visual symptoms might include visual loss, visual inattention, visual illusions and visual hallucinations. It is essential that the symptoms progress to cortical blindness. Cases with other onsets that progress to include cortical blindness are NOT included in this category.
Psychiatric Onset	These cases present with psychiatric symptoms such as depression, anxiety, paranoia and delusions, without the presence of other features for a period of at least four weeks. Non-specific malaise or apathy do not count unless accompanied by some of the above symptoms. Visual or auditory hallucinations alone do not count, but may accompany the above features. It may be difficult to distinguish between the early features of dementia and a more specifically psychiatric onset. Behavioural change straightforwardly due to a developing dementia is not included in this category. The essential characteristics of this presentation is that the patients present with a disturbance that suggests a psychiatric disturbance rather than an obvious dementia and that specifically neurological features are absent.
Cerebellar Onset	The presentation is with a progressive cerebellar syndrome without other significant features, for at least two weeks.
Extrapyramidal Onset	The presentation is with an extrapyramidal syndrome involving Parkinsonian features with or without chorea, athetosis or dystonia, but without other significant features for at least two weeks.
Stroke-like Onset	The presentation is abrupt enough for a diagnosis of stroke to be entertained in the initial stages.
Other	None of the above described presentations is applicable.
Missing	There is no clear clinical information available or the information does not allow a definite classification.

2.6 Clinical Criteria for Sporadic CJD Diagnosis

The following is adapted from the EuroCJD diagnostic criteria 2009 and was used thoughout this study to classify cases of suspected sporadic CJD[113].

2.6.1 Definite (1.0S)

Neuropathologically confirmed sporadic CJD

2.6.2 Probable (2.0S)

Rapidly progressive dementia ***AND*** at least two out of the following four clinical features:

Myoclonus
Visual or cerebellar signs
Extrapyramidal or pyramidal signs
Akinetic Mutism

AND a positive result on at least one of the following investigations:

A typical EEG (periodic sharp wave complexes)
A positive 14-3-3 CSF result in patients with a disease duration of less than two years
MRI brain high signal abnormality in the caudate nucleus and/or putamen on DWI or FLAIR imaging sequences

2.6.3 Possible (3.0S)

Rapidly progressive dementia and at least two of the following four clinical features:

Myoclonus

Visual or cerebellar signs
Pyramidal/extrapyramidal signs
Akinetic mutism

AND the absence of a positive result for any of the three investigations that would classify the case as probable (see above) ***AND*** disease duration less than two years.

2.6.4 Uncertain (4.1S)

Patients were classified as 4.1S if the case did not meet the WHO diagnostic criteria but in whom sCJD was still being considered a possibility. Examples of this include patients who have clinical phenotype that follows a longer incubation period and therefore the relevant clinical symptoms and signs have not yet evolved.

2.6.5 Non-CJD Case based on clinical evidence (4.2S)

This classification applied to cases in whom the patient did not meet diagnostic criteria and where there was strong evidence clinically to support an alternative neurological condition.

2.6.6 Non-CJD Case based on pathological evidence (4.3S)

Applied to patients where there was neuropathological confirmation of an alternative diagnosis

2.7 Statistical Analysis

Statistical analysis was performed using StatsDirect software package Version 3.1.21.

Chapter 3

Results

3 Results

Between the 1st of January 2012 and the 30th of June 2013 inclusive, 192 of clinically suspected cases of CJD were notified to the NCJDRSU. 30 of these cases were excluded either because they were notified out with the data period or because they were confirmed genetic or iatrogenic cases at notification. The exclusion criteria are detailed in the methodology section. 162 cases of clinically suspected CJD were included in this study.

3.1 Data Overview: case demographics

The median age at symptom onset was 67.1 years (range 43 to 87 years). The median duration of illness from symptom onset to death was calculated in 143 (88.3%) and was 6.3 months (range 0.69 to 42.5 months). Duration of illness could not be calculated in 19 (11.7%) due to missing date of symptom onset (n= 2), missing date of death (n= 3) and cases that are still alive (n= 14). The overall sex distribution is shown in Figure 1.

Figure 1 Overall Sex Distribution

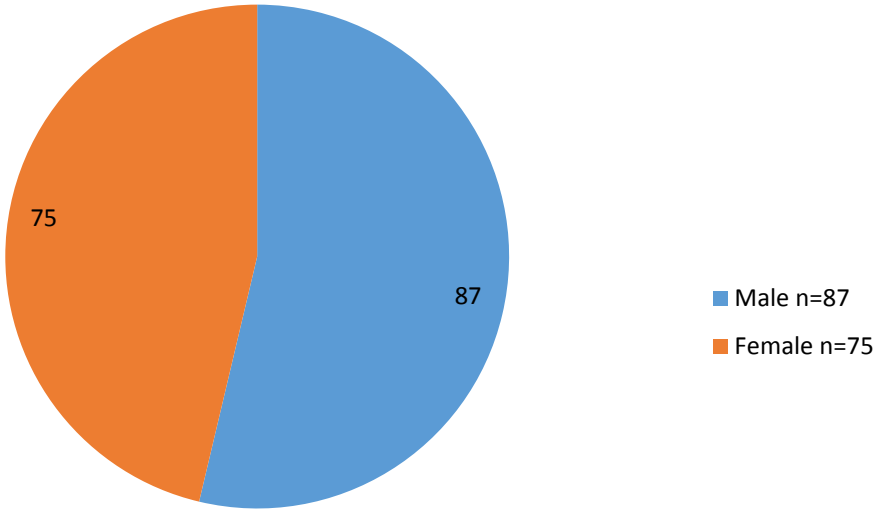
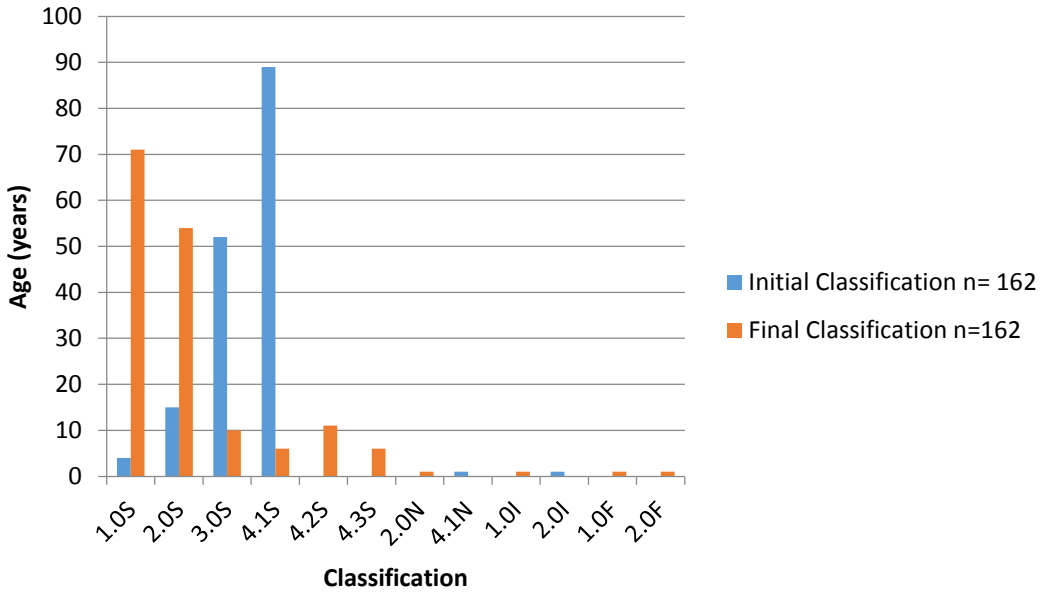


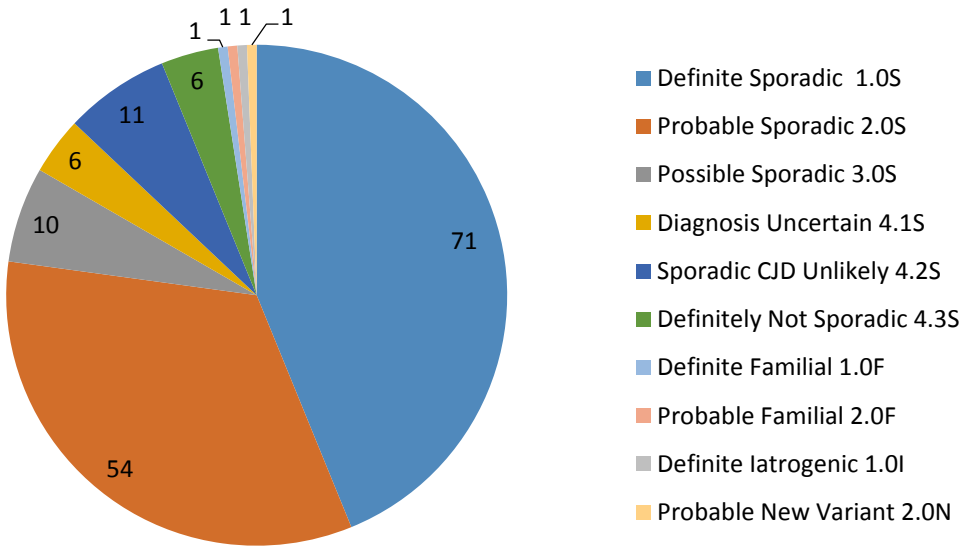
Figure 2 illustrates the initial and final classification of each case according to the EuroCJD diagnostic criteria[113]. The details of the classification criteria are given in the methodology section.

Figure 2 Classification using EuroCJD Criteria



The majority of initial classifications were either 3.0S (possible sporadic CJD) or 4.1S (diagnosis uncertain but CJD still considered to be a possibility). The majority of final classifications were either 1.0S (definite sporadic CJD) or 2.0S (probable sporadic CJD) as illustrated in Figure 2 and Figure 3. 10 cases received a final classification of 3.0S. Of these, 7 were initially classified as 4.1S and 3 as 3.0S. All 6 cases that were given a final classification of 4.1S were initially classified as 4.1S. 11 cases were given a final classification of 4.2 (sporadic CJD unlikely). 2 of these were initially classified as 3.0S and 9 classified as 4.1S. 6 cases received a final classification of 4.3 (definitely not sporadic CJD). 3 were initially classified as 3.0S and 3 as 4.1S. 1 case was initially classified as 4.1N where the diagnosis was uncertain, although there was a suspicion of new variant CJD. The final classification for this case was 2.0N (probable new variant CJD). 2 cases received a final classification of 1.0F (definite familial CJD) and 2.0F (probable familial CJD), both of which were initially classified as 4.1S. 1 case received a final diagnosis of 1.0I (definite iatrogenic CJD) and was initially classified as 2.0I (probable iatrogenic CJD).

Figure 3 Final Classification n=162



The mode of onset for each case is detailed in Table 5. The definitions of each mode of onset were defined by the EuroCJD criteria and are detailed in the methodology section. All cases that had RT-QuIC analysis have been included for comparison (n=115; 71%). Fisher's exact statistical analysis was employed to determine whether there were any statistically significant differences in the mode of onset between cases who did and did not have RT-QuIC. There were no statistically significant differences found.

Table 5 Mode of Onset

Mode of Onset	Total Cases N=162	Cases Tested for RT-QuiC N=115	Cases not tested for RT-QuIC N=47	P Value
Rapidly Progressive Dementia	74 (45.7%)	49 (42.6%)	25	0.2295
Slowly progressive Dementia	2 (1.2%)	2 (1.2%)	0	>0.9999
Stroke-Like Onset	3 (1.9%)	2 (1.2%)	1	>0.9999
Psychiatric Onset	11 (6.8%)	8 (7.0%)	3	>0.9999
Other	46 (28.4%)	36 (31.3%)	10	0.2505
Heidenhain	9 (5.6%)	4 (3.5%)	5	0.1228
Cerebellar	16 (9.9%)	13 (11.3%)	3	0.4019
Data Missing	1 (0.6%)	1 (0.9%)	0	-

As detailed in the methodology section, the 'Other' mode of onset, were cases that could not be categorised by the more common presenting features. Examples include limb pain, status epilepticus, insomnia and depression, isolated speech disturbance, systemic symptoms such as lethargy and weight loss as we all as personality change.

Table 6 shows the median age for each final classification for all cases.

Table 6 Median age for each classification n=162

Final Classification	Total No. of Cases	No. of cases with known age at onset	Median Age at Onset (years)	Age Range (years)
Definite Sporadic 1.0S	71	71 (100%)	67.5	43-81
Probable Sporadic 2.0S	54	54 (100%)	66.8	45-86
Possible Sporadic 3.0S	10	10 (100%)	68.3	58-76
Diagnosis Uncertain 4.1S	6	6 (100%)	62.3	48-75
Sporadic CJD Unlikely 4.2S	11	11 (100%)	69.4	52-87
Definitely not Sporadic CJD 4.3S	6	6 (100%)	73.2	63-85
Definitely Genetic CJD 1.0F	1	1 (100%)	55.0	-
Probable Genetic CJD 2.0F	1	1 (100%)	70.0	-
Definite Iatrogenic CJD 1.0I	1	1 (100%)	46.0	-
Probable New Variant CJD 2.0N	1	1 (100%)	46.0	-
Total	**162**	**162 (100%)**	**67.1**	**43-87**

Table 7 demonstrates the sex distribution within each final classification.

Table 7 Sex distribution for each final classification n=162

Final Classification	Total Number of Cases	Female (%)	Male (%)
Definite Sporadic 1.0S	71	36 (50.7)	35 (49.3)
Probable Sporadic 2.0S	54	27 (50.0)	27(50.0)
Possible Sporadic 3.0S	10	2 (20.0)	8 (80.0)
Diagnosis Uncertain 4.1S	6	1 (16.7)	5 (83.3)
Sporadic CJD Unlikely 4.2S	11	6 (54.5)	5 (45.4)
Definitely not Sporadic CJD 4.3S	6	1 (16.7)	5 (83.3)
Definite Genetic CJD 1.0F	1	1 (100.0)	-
Probable Genetic CJD 2.0F	1	1 (100.0)	-
Definite Iatrogenic CJD 1.0I	1	-	1 (100.0)
Probable New Variant CJD 2.0N	1	-	1 (100.0)
Total	**162**	**75**	**87**

Table 8 demonstrates the median disease duration for each final classification for all cases.

Table 8 Median disease duration for each final classification n=162

Final Classification	Total Number of Cases	No. of cases with known/calculated disease duration	Median disease duration (months)	Disease duration range (months)
Definite Sporadic 1.0S	71	69	6.2	1.1-32.9
Probable Sporadic 2.0S	54	53	4.7	0.7-15.3
Possible Sporadic 3.0S	10	8	8.8	1.5-31.7
Diagnosis Uncertain 4.1S	6	5	21.3	7.4-42.5
Sporadic CJD Unlikely 4.2S	11	0	-	-
Definitely not sporadic 4.3S	6	4	7.1	1.2-19.7
Definite Genetic CJD 1.0F	1	1	2.8	-
Probable Genetic CJD 2.0F	1	1	3.5	-
Definite Iatrogenic CJD 1.0I	1	1	4.3	-
Probable New Variant CJD 2.0N	1	1	11.6	-
Total	**162**	**143**	**6.31**	**0.7-42.5**

Disease duration could be calculated in 69 (97.2%) of the cases that were classified as 1.0S. The date of disease onset was not available for one case and date of death not available for the other. Neither of these cases had RT-QuIC performed. A value could not be calculated in one 2.0S case as the patient is still alive. For those cases that received a final classification of 3.0S, disease duration could not be calculated in 2 (20%). Both cases were still alive at the time of writing. Disease duration could not be calculated in 1 of the cases that received a final classification of 4.1S. This case was a UK resident but returned to their native Africa and so it remains unclear as to whether this case is still alive. Disease duration could not be calculated in any of the 4.2S cases as 9 are still alive and data is missing for 2 other cases. The disease duration for those classified as 4.3S could not be calculated in 2 cases as the date of onset was unknown in one case and the other is still alive.

3.2 Data Overview: Investigations

The current EuroCJD diagnostic criteria internationally employed to diagnose CJD is detailed in the methodology section. It encompasses the clinical features in association with investigation results that include MRI, EEG, CSF 14-3-3 and neuropathological examination of brain tissue. The following section provides an overview of the available data collected on each of these investigations for the entire cohort. Additional relevant information such as codon 129 is also included. Table 9 demonstrates the available MRI data for the entire cohort of patients, n=162.

Table 9 Neuroimaging: what proportion of cases underwent a MRI?

N= 162	**MRI Performed**	**MRI Sent to NCJDRSU**	**MRI Reviewed at NCJDRSU**	**MRI Positive According to NCJDRSU**	**MRI Positive According to European CJD Criteria** (also reviewed by NCJDRSU)	**MRI Positive According to Local Team Radiologist** (not reviewed by NCJDRSU**)**
Yes	154	96	95	70	57	14
No	8	58	1	25	38	42
Data Missing	-	-	-	-	-	2
Total	**162**	**154**	**96**	**95**	**95**	**58**

154/162 (95.1%) cases had a MRI performed. 96/154 (62.3%) of those who had a MRI performed had copies of the imaging sent to the NCJDRSU for review. Of these, 1 could not be assessed due to problems with software compatibility. Of those that had a MRI performed but not sent to the NCJDRSU for assessment, the results of the MRI were missing in 2 cases. As detailed in the methodology section, consultant neuro-radiologist, Dr David Summers based classified the imaging according to the EuroCJD diagnostic criteria, but also gave an overall opinion based on clinical experience, whether the scan was radiologically suggestive of sporadic CJD. This included scans that demonstrated isolated cortical ribboning which is the explanation as to why the number of positive scans differs between the EuroCJD diagnostic criteria and the NCJDRSU.

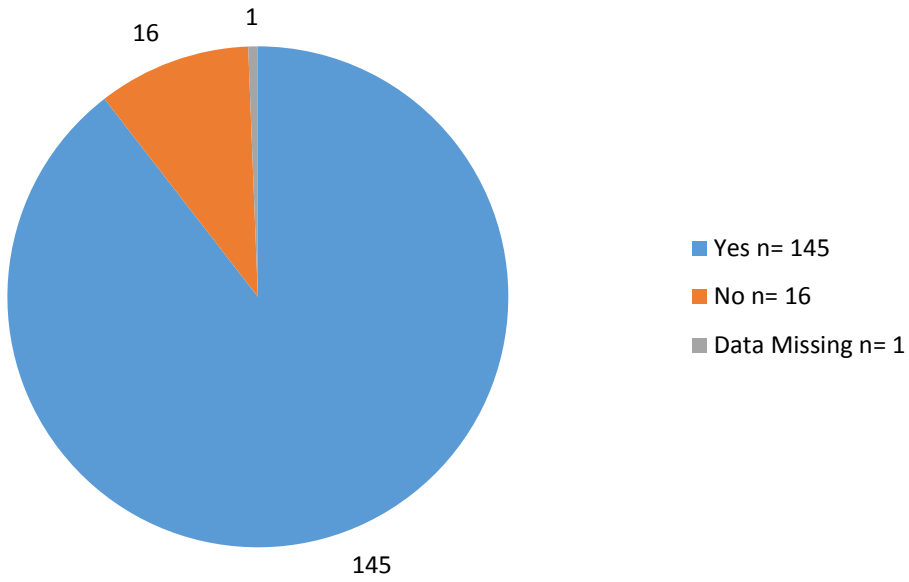
Table 10 shows the number of cases that had an EEG performed during their illness and what proportion of these had a copy of the representative pages of the EEG reviewed at the NCJDRSU.

Table 10 Electroencephalogram: what proportion of cases underwent an EEG?

N=162	EEG Performed	EEG Sent to NCJDRSU	EEG Reviewed at NCJDRSU	EEG Positive According to NCJDRSU	EEG Positive According to Referring Hospital (not reviewed by NCJDRSU)
Yes	150	91	91	29	15
No	6	59	0	62	43
Data Missing	6	-	-	-	1
Total	**162**	**150**	**91**	**91**	**59**

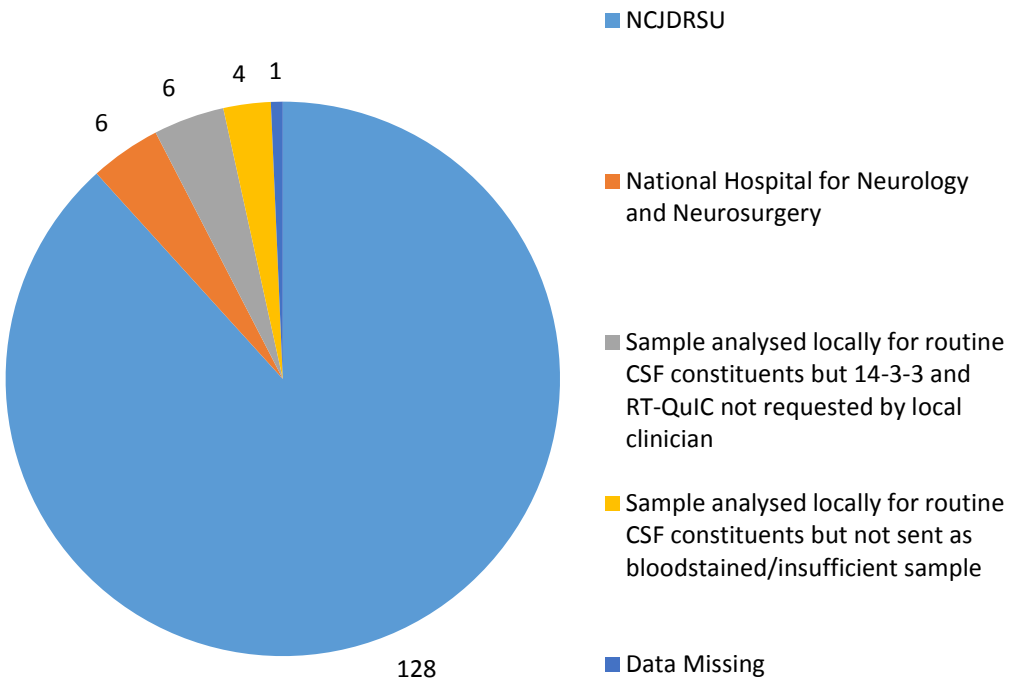
An EEG was performed in 150 cases in total. Of these, 91 (60.7%) were sent to the NCJDRSU and all were reviewed. 59 (39.3%) were not sent to the NCJDRSU for assessment and the local report is shown for these. Data was missing in 1 case. Figure 4 illustrates the proportion of the cohort who underwent a lumbar puncture and subsequent cerebrospinal fluid analysis.

Figure 4 Proportion of cases that underwent cerebrospinal fluid analysis



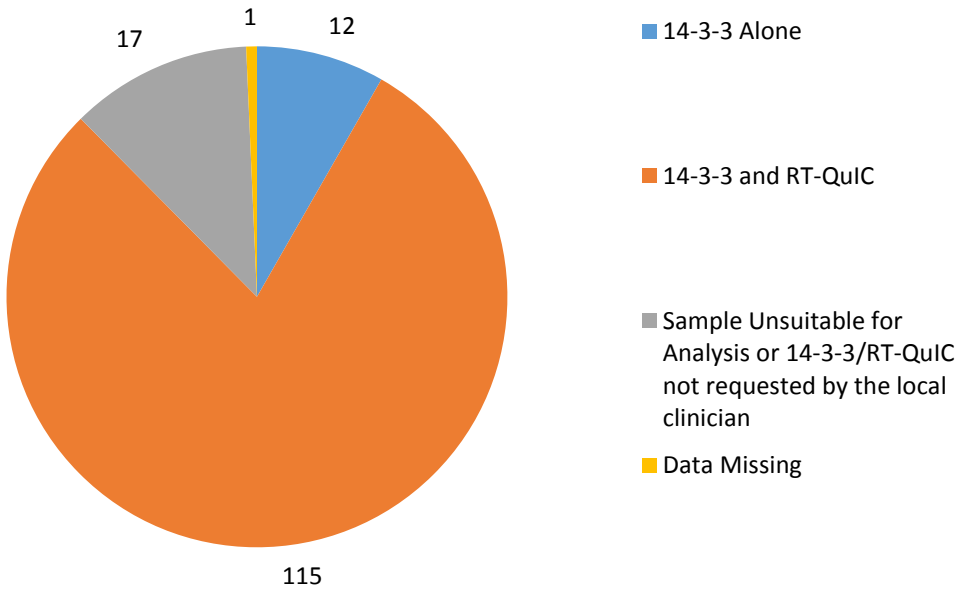
145/162 (89.5%) of the study cohort had a lumbar puncture. Figure 5 illustrates the proportion of cerebrospinal fluid samples that were analysed at the NCJDRSU. A proportion of cases were discussed with the NCJDRSU for 14-3-3 and RT-QuIC analysis but samples were not sent as they were unsuitable due to insufficient volume or bloodstained. Other samples were analysed locally for routine CSF constituents but the local clinicians did not refer for 14-3-3 and RT-QuIC. The remaining samples were analysed at the National Hospital for Neurology and Neurosurgery in London, although this was only for 14-3-3.

Figure 5 Proportion of CSF samples analysed at NCJDRSU n=128



128/145 samples of CSF were received by the NCJDRSU of which 121 were analysed for 14-3-3 +/- RT-QuIC, as shown in Figure 5. The remaining 7 samples could not be analysed as they were found to be of insufficient volume or because they were bloodstained.

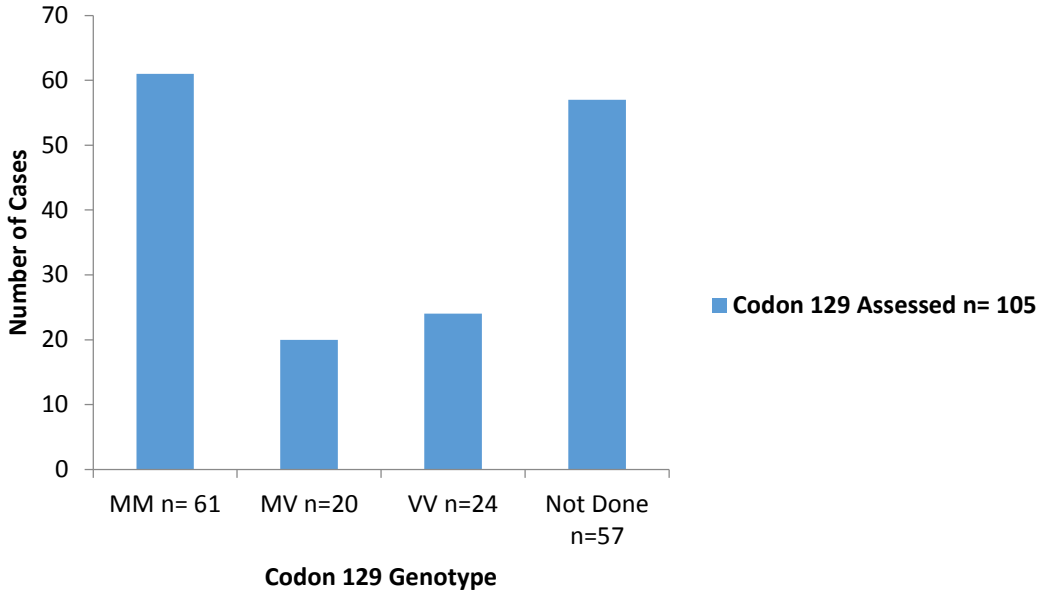
Figure 6 Proportion of CSF samples analysed for RT-QuIC and 14-3-3 n=115



12/145 cases were analysed for 14-3-3 and not RT-QuIC. 6/12 of these samples were analysed at the National Hospital for Neurology and Neurosurgery and we did not receive a sample. 6/12 cases were analysed by the NJCDRSU but there was not enough CSF for RT-QuIC to be performed in addition to 14-3-3.

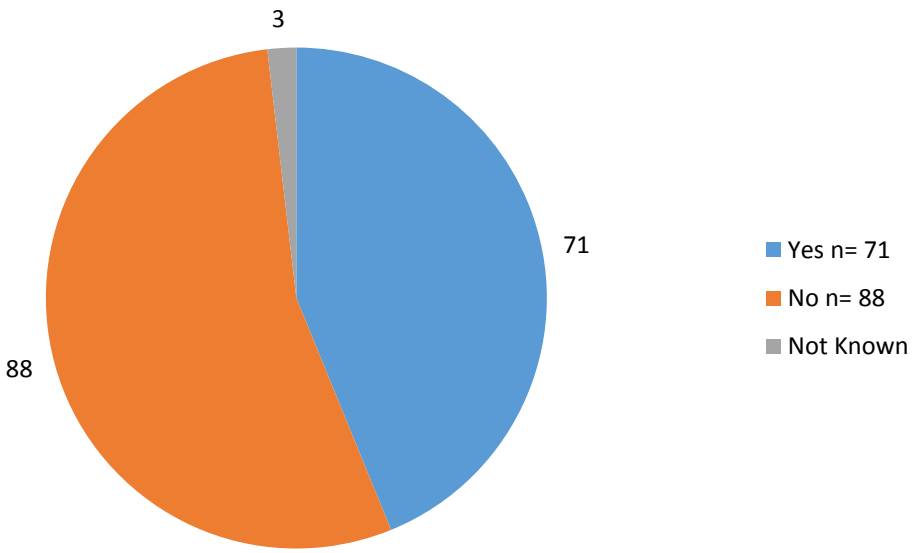
The proportion of cases that had codon 129 analysis is illustrated in Figure 7. A total of 105/162 (64.8%) were analysed for the codon 129 polymorphism.

Figure 7 Proportion of cases with codon 129 analysis n=162



The proportion of cases that underwent a post mortem and subsequent neuro-pathological analysis is shown in Figure 8.

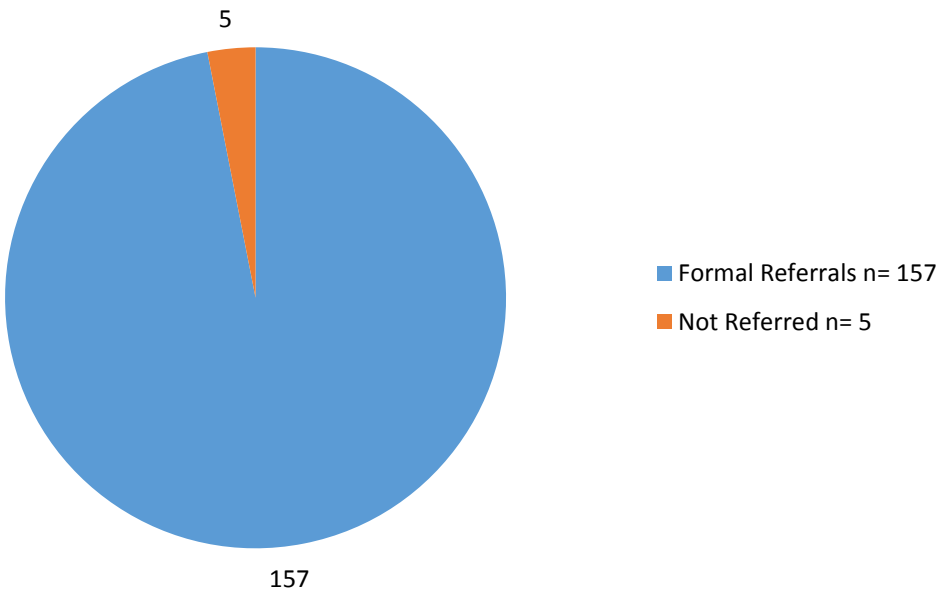
Figure 8 Proportion of cases in whom a post mortem was performed with subsequent neuropathological assessment



3.3 Case Referrals

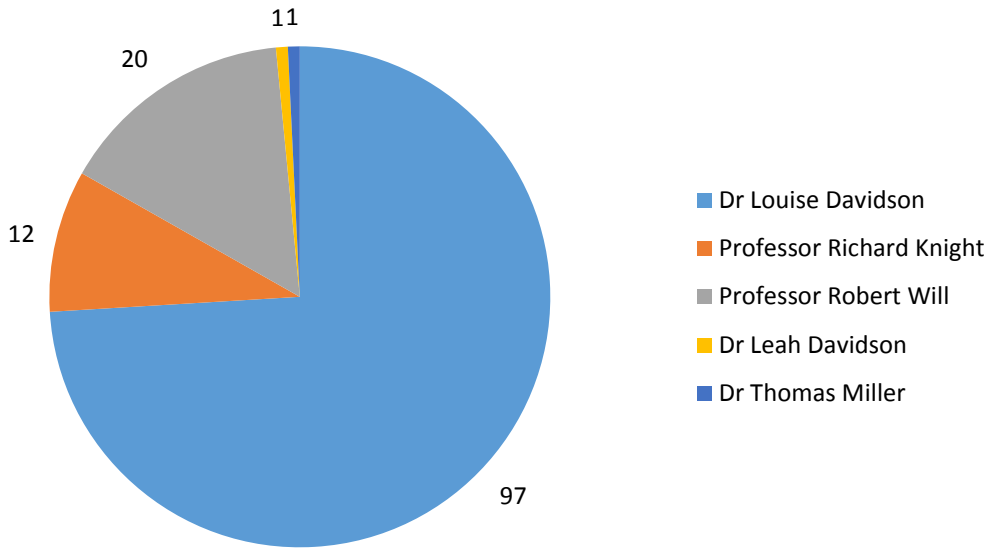
The proportion of cases formally referred to the NCJDRSU is illustrated in Figure 9.

Figure 9 Proportion of cases formally referred to NCJDRSU n=162



157 (96.9%) of the study cohort were formally referred to the NCJDRSU and 5 (3.08%) were not. Of the non-referred cases, the offer of a visit was declined in two cases by the local clinicians. One case was genetic and the remaining two cases were for discussion only. 131 were referred and reviewed during life. 7 cases were reviewed after death. 19 cases were formally referred after death but not reviewed during the study period. The families of 3 of the 19 cases declined a visit. For those cases that were reviewed either during life or after death, the assessing clinician is illustrated in Figure 10 and shows that the author reviewed the majority of the cases.

Figure 10 Clinicians who reviewed formally referred cases



For the purposes of this study, only the clinical characteristics of suspected cases of sporadic CJD with a RT-QuIC result will be analysed.

3.4 Clinical Characteristics of Definite Sporadic (1.0S) RT-QuIC positive and RT-QuIC negative cases

The proportion of the cohort with a final classification of 1.0S was 43.8% (n=71). The total number of 1.0S cases who had cerebrospinal fluid analysis was n=60. Data was missing in one case. The number of cases with a final classification of 1.0S that had cerebrospinal fluid successfully analysed for both 14-3-3 and RT-QuIC was n=44. There were no cases where RT-QuIC was successfully analysed without 14-3-3. The following series of tables compare the mean age of onset, median duration of illness, mode of symptom onset, codon 129 genotype, MRI, EEG and 14-3-3 results between the RT-QuIC positive and negative groups.

Table 11 demonstrates the mean age of onset, median duration of illness and mode of symptom onset between the 1.0S RT-QuIC positive and RT-QuIC negative groups. Logistical regression analysis was used to assess the predictive value of age and disease duration on a positive RT-QuIC result. Odds ratios with their corresponding P values are provided. A longer duration of illness was more likely to be associated with a negative RT-QuIC result and this was found to be significant. The age of onset within the positive group was relevant in the positive RT-QuIC group but this did not reach statistical significance.

Table 11 Mean age at onset, median duration of illness and mode of onset

	RT-QuIC Positive n= 35	**RT-QuIC Negative n= 9**	**Odds Ratio**	**P Value**
Mean Age at Onset (Years)	66.6 (range 43-78)	61.1 (range 51-71)	1.023	0.644
Median Duration of Illness (months)	4.47 (range 1.1-19.9)	13.0 (range 2.2-32.4)	0.82	0.014
Mode of Symptom Onset	-	-	-	-
Rapidly Progressive Dementia	16 (45.7%)	4 (44.4%)	-	-
Slowly Progressive Dementia	0 (0%)	0 (0%)	-	-
Heidenhain	2 (5.7%)	1 (11.1%)		
Cerebellar	4 (11.4%)	0 (0%)		
Stroke-Like Onset	1 (2.9%)	0 (0%)		
Psychiatric Onset	2 (5.7%)	0 (0%)		
Other	10 (28.5%)	4 (44.4%)		

The codon 129 status was known for 40/44 (90.9%) of the 1.0S cases with a RT-QuIC result, the details of which are shown in Table 12 below. Logistic regression analysis was used to assess the predictive value of each genotype in producing a positive RT-QuIC result. Odds ratios with corresponding P values are shown. Codon 129 MM and MV were more likely to produce a positive result than VV genotype, although neither of these reached statistical significance. The specific isotype was known in 23/44 (52.3%) of cases and is illustrated in Table 13.

Table 12 Codon 129 status for definite (1.0S) RT-QuIC positive and RT-QuIC negative cases n=40

	RT-QuIC Positive n=35	**RT-QuIC Negative n= 9**	**Odds Ratio**	**P Value**
Codon 129 Analysed	**31/35 (88.5%)**	**9/9 (100%)**		
MM	18 (58.0)	3 (33.3%)	1.90	0.531
MV	6 (19.3%)	2 (22.2%)	6.05	0.301
VV	7 (22.5%)	4 (44.4%)	0.69	0.745
Total	**31**	**9**		

Table 13 Codon 129/Isotype for definite (1.0S) Rt-QuIC positive and RT-QuIC negative cases n=44

	RT-QuIC Positive n= 35	**RT-QuIC Negative n= 9**
Isotype Analysed	**19/35 (54.2%)**	**4/9 (44.4%)**
MM1	9 (47.3%)	0 (0%)
MV1	3 (15.7%)	0 (0%)
VV1	0 (0%)	1 (25%)
MM2A	0 (0%)	1 (25%)
MV2A	1 (5.2%)	1 (25%)
VV2A	4 (21.0%)	0 (0%)
MM mixed	2 (10.5%)	0 (0%)
MV mixed	0 (0%)	1 (25%)
VV mixed	0 (0%)	0 (0%)
Total	**19**	**4**

Table 14 Neuroimaging: MRI assessment for definite (1.0S) RT-QuIC positive and RT-QuIC negative cases n=44

	RT-QuIC Positive n= 35	**RT-QuIC Negative n= 9**
MRI Performed	**31/35 (88.5%)**	**9/9 (100%)**
MRI Sent to NCJDRSU	**20/31 (64.5%)**	**8/9 (88.8%)**
MRI Reviewed at NCJDRSU	**20/31 (64.5%)**	**8/9 (88.8%)**
MRI Positive According to NCJDRSU		
Yes	16/20 (80%)	5/8 (12.5%)
No	4/20 (20%)	3/8 (37.5%)
Basal Ganglia change only	0/16 (0%)	2/5 (40%)
Basal ganglia change + Cortical Ribboning	15/16 (93.7%)	3/5 (60%)
Cortical Ribboning Alone	1/16 (6.25%)	0/5 (0%)
MRI Positive According to WHO Criteria (also reviewed at NCJDRSU)		
Yes	15/20 (75%)	5/8 (12.5%)
No	5/20 (25%)	3/8 (37.5%)
Basal Ganglia change only	0/15 (0%)	2/5 (40%)
Basal ganglia change + Cortical Ribboning	15/15(100%)	3/5 (60%)
Cortical Ribboning Alone	0/15 (0%)	0/5 (0%)
MRI Positive According to WHO Criteria (not reviewed at NCJDRSU)	**11/31 (35.4%)**	**1/9 (11.1%)**
Yes	4/11 (36.3%)	0/1 (0%)
No	6/11 (54.5%)	1/1 (100%)
Data Missing	1/11 (9.09%)	-
Basal Ganglia Alone	2/4 (50%)	0/1 (0%)
Basal Ganglia + Cortical Ribboning	2/4 (50%)	0/1 (0%)
Cortical Ribboning Alone	0/4 (0%)	1/1 (100%)

Table 15 EEG assessment for definite (1.0S) RT-QuIC positive and RT-QuIC negative cases

	RT-QuIC Positive n= 35	**RT-QuIC Negative n= 9**
EEG Performed		
Yes	**33/35 (94.2%)**	**9/9 (100%)**
No	**0/35 (0%)**	**0/9 (0%)**
Data Missing	**2/35 (5.71%)**	**0/9 (0%)**
EEG Reviewed at NCJDRSU	**21 (63.6%)**	**5 (55.5%)**
Positive	8 (38.1%)	1 (20%)
Negative	13 (61.9%)	4 (80%)
EEG Reviewed Locally	**12 (36.3%)**	**4 (44.4%)**
Positive	4 (33.3%)	1 (25%)
Negative	8 (66.6%)	3 (75%)

Table 16 Comparison of 14-3-3 and RT-QuIC results for definite (1.0S) cases

	RT-QuIC Positive n= 35	**RT-QuIC Negative n =9**
Median Time from Symptom Onset to LP (range in months)	3.22 (0.62-9.4)	6.81 (0.56-13.4)
14-3-3		
Positive	31(94.2%)	5 (55.5%)
Negative	4 (11.4%)	4 (44.4%)

The median time to LP was calculated in 34/35 RT-QuIC positive cases and 9/9 RT QuIC negative cases. Date of lumbar puncture was not available in one case. The predictive value of the duration from symptom onset to LP on the RT-QuIC result was analysed using logistic regression. This produced an odds ratio of 0.72 suggesting that there is no influence of the timing of the LP on the RT-QuIC result.

This finding was significant (p= 0.0094). The agreement between 14-3-3 and RT-QuIC was assessed using Cohen's Kappa returning a value 0.33 suggesting a fair agreement between the two tests (p= 0.011).

3.5 Clinical Characteristics of Probable Sporadic (2.0S) RT-QuIC positive and RT-QuIC negative cases

The proportion of the study cohort with a final classification of 2.0S was n=54. The number of cases that had cerebrospinal fluid analysis was 50/54 (93%). Of these, the proportion of cases that had both 14-3-3 and RT-QuIC analysed was n=45. Table 17 illustrates the median age of onset, median duration of illness and mode of symptom onset for both 2.0S RT-QuIC positive and RT-QuIC negative cases. Logistical regression was used to assess the predictive value of age and duration of illness on the RT-QuIC result. Odds ratios and corresponding P values are shown. Older age and shorter duration of illness were shown to be more likely to produce a positive result although statistical significance was not reached.

Table 17 Mean age of onset, median duration of illness and mode of onset for probable (2.0S) RT-QuIC positive and RT-QuIC negative cases n=54

	RT-QuIC Positive n= 38	**RT-QuIC Negative n= 7**	**Odds Ratio**	**P Value**
Mean Age at Onset (Years)	**70 (range 45-84)**	**66.9 (range 47-86)**	**1.023**	**0.628**
Median Duration of Illness (months)	**4.48 (range 0.69-11.9)**	**3.57 (range 1.64-6.1)***	**1.33**	**0.274**
Mode of Symptom Onset	-	-	-	-
Rapidly Progressive Dementia	19 (50%)	2 (28.5%)	-	-
Slowly Progressive Dementia	0 (0%)	0 (0%)	-	-
Heidenhain	1 (2.6%)	0 (0%)	-	-
Cerebellar	6 (15.7%)	1 (14.2%)		
Stroke-Like Onset	0 (0%)	1 (14.2%)		
Psychiatric Onset	2 (5.3%)	2 (28.5%)		
Other	9 (23.6%)	1 (14.2%)		
Data Missing	1 (2.6%)	0 (0%)		

*The median duration of illness could not be calculated in one case as the patient was still alive at the time of writing.

Table 18 illustrates the *PRNP* codon 129 genotype..

Table 18 Codon 129 status for probable (2.0S) RT-QuIC positive and RT-QuIC negative cases

	RT-QuIC Positive n=38	**RT-QuIC Negative n=7**
Codon 129 Analysed	**17/38 (44.7%)**	**2/7 (28.6%)**
MM	9 (52.9%)	1 (50%)
MV	1 (5.9%)	0 (0%)
VV	7 (41.2%)	1 (50%)
Total	**17**	**2**

Codon 129/Isotype for probable sporadic (2.0S) RT-QuIC Cases

Not performed in either group due to absence neuropathological examination.

Table 19 Neuroimaging: MRI assessment of probable (2.0S) RT-QuIC positive and RT-QuIC negative cases

	RT-QuIC Positive n= 38	**RT-QuIC Negative n= 7**
MRI Performed	**38/38 (100%)**	**7/7 (100%)**
MRI Sent to NCJDRSU	**25/38 (65.8%)**	**5/7 (71.4%)**
MRI Reviewed at NCJDRSU	**25/38 (65.8%)**	**4/7 (57.1%)**
MRI Positive According to NCJDRSU		
Yes	21/25 (84%)	2/4 (50%)
No	4/25 (16%)	2/4 (50%)
Basal Ganglia change only	3/21 (14.3%)	2/2 (100%)
Basal ganglia change + Cortical Ribboning	16/21 (76.2%)	0/2 (0%)
Cortical Ribboning Alone	2/21 (9.5%)	0/2 (0%)
MRI Positive According to WHO Criteria (also reviewed at NCJDRSU)		
Yes	**19/25 (76%)**	**2/4 (50%)**
No	**6/25 (24%)**	**2/4 (50%)**
Basal Ganglia change only	3/19 (15.8%)	2/2 (100%)
Basal ganglia change + Cortical Ribboning	16/19 (84.2%)	0/2 (0%)
Cortical Ribboning Alone	0/19 (0%)	0/2 (0%)
MRI Positive According to WHO Criteria (not reviewed at NCJDRSU)		
Yes	**5/13 (38.5%)**	**1/3 (33.3%)**
No	**7/13 (53.8%)**	**2/3 (66.7%)**
Data Missing	**1/13 (7.7%)**	-
Basal Ganglia Alone	0/5 (0%)	1/1 (100%)
Basal Ganglia + Cortical Ribboning	5/5 (100%)	0/1 (0%)
Cortical Ribboning Alone	0/5 (0%)	0/1 (0%)
Data Missing	1/13 (7.7%)	-

Table 20 EEG assessment of probable (2.0S) RT-QuIC positive and RT-QuIC negative cases

	RT-QuIC Positive n=38	**RT-QuIC Negative n=7**
EEG Performed		
Yes	**36/38 (94.7%)**	**6/7 (85.7%)**
No	**2/38 (5.3%)**	**1/7 (14.3%)**
EEG Reviewed at NCJDRSU	**23/36 (63.9%)**	**4/6 (66.7%)**
Positive	6/23 (26.1%)	2 (50%)
Negative	17/23 (73.9%)	2 (50%)
EEG Locally Reported	**13/36 (36.1%)**	**2/6 (33.3%)**
Positive	5/13 (38.5%)	1 (50%)
Negative	8/13 (61.5%)	1 (50%)

Table 21 Comparison of 14-3-3 and RT-QuIC for probable (2.0S) cases

	RT-QuIC Positive n= 38	**RT-QuIC Negative n =7**
Median time from symptom onset to LP (months)	3.48 (range 0.36-11.4)	3.85 (range 1.08-13.1)
14-3-3		
Positive	34/38 (89.5%)	6/7 (85.7%)
Negative	4/38 (10.5%)	1/7 (14.3%)

The influence of the duration between symptom onset and LP on producing a positive RT-QuIC result was analysed using logistic regression. The odds ratio was 0.93 suggesting that the timing of the lumbar puncture does not influence the outcome of the result although this finding was not significant (p= 0.632). The agreement between 14-3-3 and RT-QuIC was analysed using Cohen's Kappa returning a Kappa value of 0.04 suggesting a poor agreement between the two tests, although this was not found to be a significant result (p- 0.383).

3.6 Clinical Characteristics of possible sporadic (3.0S) RT-QuIC positive and negative cases

The number of cases who received a final classification of 3.0S at the time of writing was n=10. The proportion of these cases that underwent a lumbar puncture and subsequent cerebrospinal fluid analysis was 9/10 (90%). Of these, the number of cases who had both 14-3-3 and RT-QuIC analysed was 5/9 (55.6%). The clinical details and investigation results for these cases are shown in the series of tables below (Tables 22-24 inclusive).

Logistical regression was used to assess the predictive value of age and duration of illness on the likelihood of a positive result. No significant findings were observed, although it is recognised by the author that the disease duration could not be calculated in the RT-QuIC negative group and that the case numbers within this diagnostic group are small.

Table 22 Mean age at onset, median duration of illness, mode of onset for possible (3.0S) RT-QuIC positive and RT-Quic negative cases n=5

	RT-QuIC Positive n= 3	**RT-QuIC Negative n= 2**	**Odds Ratio**	**P Value**
Mean Age at Onset (Years)	**67 (no range)**	**64 (range 58-70)**	**77.32**	**0.999**
Median Duration of Illness (months)	**4.26 (range 1.51-6.46)**	*****	1	**>0.999**
Mode of Symptom Onset	**-**	**-**	**-**	**-**
Rapidly Progressive Dementia	1/3 (33.3%)	1/2 (50%)		
Slowly Progressive Dementia	0 (0%)	0 (0%)		
Heidenhain	0 (0%)	0 (0%)		
Cerebellar	1/3 (33.3%)	0 (0%)		
Stroke-Like Onset	0 (0%)	0 (0%)		
Psychiatric Onset	0 (0%)	0 (0%)		
Other	1/3 (33.3%)	1/2 (50%)		
Data Missing	0 (0%)	0 (0%)		

*The median disease duration could not be calculated. The disease duration of one case was 11.5 months. The other case, to date, is still alive. The date of symptom onset for this case was 15th November 2011.

Table 23 Codon 129 status in the possible (3.0S) RT-QuiC positive and RT-QuIC negative group

	RT-QuIC Positive n=3	**RT-QuIC Negative n=2**
Codon 129 Analysed	**3/3 (100%)**	**2/2 (100%)**
MM	2/3 (66.7%)	0 (0%)
MV	0 (0%)	1/2 (50%)
VV	1/3 (33.3%)	1/2 (50%)
Total	**3**	**2**

Codon 129/Isotype for the possible sporadic (3.0S) RT-QuIC Positive and RT-QuIC Negative Cases

Not performed in either group.

Table 24 Neuroimaging: MRI assessment of possible (3.0S) RT-QuIC positive and RT-QuIC negative cases

	RT-QuIC Positive n= 3	**RT-QuIC Negative n= 2**
MRI Peformed	**3/3 100%)**	**2/2 (100%)**
MRI Sent to NCJDRSU	**2/3 (66.6%)**	**1/2 (50%)**
MRI Reviewed at NCJDRSU	**2/3 (66.6%)**	**1/2 (50%)**
MRI Positive According to NCJDRSU		
Yes	2/2 (100%)	1/1 (100%)
No	0/2 (0%)	0/1 (0%)
Basal Ganglia change only	0/2 (0%)	0/1 (0%)
Basal ganglia change + Cortical Ribboning	0/2 (0%)	0/1 (0%)
Cortical Ribboning Alone	2/2 (100%)	1/1 (100%)
MRI Positive According to WHO Criteria (also reviewed at NCJDRSU)		
Yes	0/2 (0%)	0/1 (0%)
No	2/2 (100%)	1/1 (100%)
Basal Ganglia change only	0 (0%)	0 (0%)
Basal ganglia change + Cortical Ribboning	0 (0%)	0 (0%)
Cortical Ribboning Alone	0 (0%)	0 (0%)
MRI Positive According to WHO Criteria (not reviewed at NCJDRSU)	1/3 (33.3%)	1/2 (50%)
Yes	0/1 (0%)	0/1 (0%)
No	1/1 (100%)	1/1 (100%)
Data Missing	-	-
Basal Ganglia Alone	0 (0%)	0 (0%)
Basal Ganglia + Cortical Ribboning	0 (0%)	0 (0%)
Cortical Ribboning Alone	0 (0%)	0 (0%)
Data Missing	-	-

Table 25 EEG assessment of possible (3.0S) RT-QuIC positive and RT-QuIC negative cases

	RT-QuIC Positive n= 3	**RT-QuIC Negative n= 2**
EEG Performed		
Yes	**2/3 (66.7%)**	**2/2 (100%)**
No	**1/3 (33.3%)**	**0 (0%)**
Data Missing	-	-
EEG Reviewed at NCJDRSU	**1/2 (50%)**	**0 (0%)**
Positive	0 (0%)	-
Negative	1/1 (100%)	-
EEG Reviewed Locally	**1/2 (50%)**	**2/2 (100%)**
Positive	0 (0%)	0 (0%)
Negative	1/1 (100%)	2/2 (100%)

Table 26 Comparison of 14-3-3 and RT-QuIC for possible (3.0S) cases

	RT-QuIC Positive n=3	**RT-QuIC Negative n=2**
Median time from symptom onset to LP (months)	3.22 (range 1.05-5.38)	4.98 (range 4.03-5.93)
14-3-3		
Positive	0 (0%)	0 (0%)
Negative	3 (100%)	2 (100%)

The predictive value of the duration between symptom onset and LP was once again analysed using logistic regression to determine whether this influences the outcome of the RT-QuIC result. The odds ratio was 0.44 suggesting that there is no influence although this finding was not found to be significant (p=0.327). The agreement between 14-3-3 and RT-QuIC was analysed using Cohen's Kappa. The value returned at 0.33 suggesting a fair agreement although this result was not significant (p= 0.078).

3.7 Clinical characteristics of Uncertain (4.1S) RT-QuIC positive and RT-QuIC negative cases

The number of cases that received a final classification of 4.1S was 6. All of these cases underwent a lumbar puncture and subsequent CSF analysis and all were assessed for 14-3-3 and RT-QuIC. The clinical characteristics and investigation findings are detailed in the series of tables below.

Logistical regression was used to determine the predictive value of age and disease duration on the likelihood of a RT-QuIC result but no statistically significant observations were made which, once again, is likely to reflect the small numbers of cases within this diagnostic group.

Table 27 Mean age of onset, median duration of illness, mode of onset for uncertain (4.1S) RT-QuIC positive and RT-QuIC negative cases n-6

	RT-QuIC Positive n= 2	**RT-QuIC Negative n= 4**	**Odds Ratio**	**P Value**
Mean Age at Onset (Years)	70 (range 67-73)	58.5 (range 48-75)	0.006	0.999
Median Duration of Illness (months)	9.1 (range 7.4-10.8)	29.5 (range 14.9-42.5)	0	0.999
Mode of Symptom Onset	-	-	-	-
Rapidly Progressive Dementia	2 (100%)	1 (20%)		
Slowly Progressive Dementia	0 (0%)	1 (20%)		
Heidenhain	0 (0%)	0 (0%)		
Cerebellar	0 (0%)	0 (0%)		
Stroke-Like Onset	0 (0%)	0 (0%)		
Psychiatric Onset	0 (0%)	3 (60%)		
Other	-	-		

*Disease duration could not be calculated in one case as the patient is still alive at the time of writing. The date of symptom onset for this case was the 15th January 2012.

Table 28 Codon 129 status for uncertain (4.1S) RT-QuIC positive and RT-QuIC negative cases

	RT-QuIC Positive n= 2	**RT-QuIC Negative n= 4**
Codon 129 Analysed	**0/2 (0%)**	**2/4 (50%)**
MM	-	1/2 (50%)
MV	-	1/2 (50%)
VV	-	0 (0%)

Codon 129/Isotype for the uncertain (4.1S) RT-QuIC Positive and RT-QuIC Negative Cases

Not performed in either group.

Table 29 Neuroimaging: MRI assessment of unccertain (4.1S) RT-QuIC positive and RT-QuIC negative cases

	RT-QuIC Positive n= 2	**RT-QuIC Negative n= 4**
MRI Performed	**2/2 (100%)**	**4/4 (100%)**
MRI Sent to NCJDRSU	**1/2 (50%)**	**4/4 (100%)**
MRI Reviewed at NCJDRSU	**1/2 (50%)**	**4/4 (100%)**
MRI Positive According to NCJDRSU		
Yes	1/1(100%)	3/4 (75%)
No	0/1 (0%)	1/4 (25%)
Basal Ganglia change only	0 (0%)	1/3 (33.3%)
Basal ganglia change + Cortical Ribboning	1/1 (100%)	1/3 (33.3%)
Cortical Ribboning Alone	0 (0%)	1/3 (33.3%)
MRI Positive According to WHO Criteria (also reviewed at NCJDRSU)		
Yes	1/1 (100%)	2/4 (50%)
No	0 (0%)	2/4 (50%)
Basal Ganglia change only	0 (0%)	1/2 (50%)
Basal ganglia change + Cortical Ribboning	1/1 (100%)	1/2 (50%)
Cortical Ribboning Alone	0 (0%)	0 (0%)
MRI Positive According to WHO Criteria (not reviewed at NCJDRSU)		
Yes	**0/1 (0%)**	**0/1 (0%)**
No	**1/1 (100%)**	**1/1 (100%)**
Data Missing	**-**	**-**
Basal Ganglia Alone	**0 (0%)**	**0 (0%)**
Basal Ganglia + Cortical Ribboning	**0 (0%)**	**0 (0%)**
Cortical Ribboning Alone	**0 (0%)**	**0 (0%)**
Data Missing	**-**	**-**

Table 30 EEG assessment of uncertain (4.1S) RT-QuIC positive and RT-QuIC negative cases

	RT-QuIC Positive n=2	**RT-QuIC Negative n=4**
EEG Performed		
Yes	2/2 (100%)	4/4 (100%)
No	0 (0%)	0 (0%)
Data Missing	-	-
EEG Reviewed at NCJDRSU	**1/2 (50%)**	**1/4 (25%)**
Positive	0 (0%)	0 (0%)
Negative	1/1 (100%)	1/4 (100%)
EEG Reviewed Locally	**1/1 (100%)**	**3/4 (75%)**
Positive	0 (0%)	0 (0%)
Negative	1/1 (100%)	3/3 (100%)

Table 31 Comparison of 14-3-3 and RT-QuIC for uncertain (4.1S) cases

	RT-QuIC Positive n= 2	**RT-QuIC Negative n =4**
Median Time to LP from Symptom Onset	**5.1 (range 4.7-5.4)**	**14.7 (range 2.5-27.3)***
14-3-3		
Positive	0 (0%)	0 (0%)
Negative	2/2 (100%)	4/4 (100%)

*Time from symptom onset to LP could not be calculated in one case as the date of the LP was missing.

Logistic regression analysis was used to assess the predictive value of the duration between symptom onset and LP on the RT-QuIC result. This produced an odds ratio of 0.87 suggesting that there is no influence although this finding was not significant, presumably due to the small numbers, p- 0.315. The agreement between 14-3-3 and RT-QuIC was also assessed using Cohen's Kappa. The Kappa value was 0.33 suggesting a fair agreement but this was not significant (p=0.0786).

3.8 Clinical characteristics of unlikely sporadic (4.2S) and definitely not sporadic (4.3S) RT-QuIC cases

The number of cases with a final classification of 4.2S was 10 and all of these had CSF analysis performed. 7/10 had both 14-3-3 and RT-QuIC performed. All were RT-QuIC negative. The mean age at onset was 74.4 years. The number of cases that received a final classification of 4.3S was 5 and all underwent CSF analysis. 4/5 had both 14-3-3 and RT-QuIC assessed. Given that both 4.2S and 4.3S classifications are considered to be not CJD, these groups have been assessed together. The following series of tables demonstrates the clinical characteristics and investigations for the 4.2S and 4.3S RT-QuIC negative cases.

Table 32 Mean age at onset, median duration of illness, mode of onset for unlikely (4.2S) and definitely not (4.3S) RT-QuIC negative cases n=12

	RT-QuIC Positive (n=0)	**RT-QuIC Negative (n=12)**
Mean Age at Onset (Years)	-	72 (range 52-87 years)
Median Duration of Illness (months)	-	9.1 (range 3.1-19.7)*
Mode of symptom onset		
Rapidly progressive dementia	-	2/12 (16.7%)
Slowly progressive dementia	-	1/12 (8.3%)
Heidenhain	-	0 (0%)
Cerebellar	-	0 (0%)
Stroke-like onset	-	0 (0%)
Psychiatric onset	-	2/12 (16.7%)
Other	-	7/12 (58.3%)
Data missing	-	0 (0%)

Table 33 Codon 129 status for unlikely (4.2S) and definitely not (4.3S) RT-QuIC negative cases

	RT-QuIC Positive n=0	**RT-QuIC Negative n=12**
Codon 129 Analysed	-	**4/12 (33.3%)**
MM	-	3/4 (75%)
MV	-	1/4 (25%)
VV	-	0 (0%)
Total	-	**4**

Codon 129/Isotype for the unlikely (4.2S) and definitely not (4.3S) RT-QuIC Positive and RT-QuIC Negative Cases

Not performed in either group.

Table 34 Neuroimaging: MRI assessment of unlikely (4.2S) and definitely not (4.3S) RT-QuIC negative cases

	RT-QuIC Positive n= 0	**RT-QuIC Negative n= 12**
MRI Performed	-	**10/12 (83.3%)**
MRI Sent to NCJDRSU	-	**7/10 (70%)**
MRI Reviewed at NCJDRSU	-	**7/7 (100%)**
MRI Positive According to NCJDRSU		
Yes	-	0/7 (0%)
No	-	7/7 (100%)
Basal Ganglia change only	-	0 (0%)
Basal ganglia change + Cortical Ribboning	-	0 (0%)
Cortical Ribboning Alone	-	0 (0%)
MRI Positive According to WHO Criteria (also reviewed at NCJDRSU)		
Yes	-	0/7 (0%)
No	-	7/7 (100%)
Basal Ganglia change only	-	0 (0%)
Basal ganglia change + Cortical Ribboning	-	0 (0%)
Cortical Ribboning Alone	-	0 (0%)
MRI Positive According to WHO Criteria (not reviewed at NCJDRSU)	-	
Yes	-	0/3 (0%)
No	-	3/3 (100%)
Data Missing	-	-
Basal Ganglia Alone	-	0 (0%)
Basal Ganglia + Cortical Ribboning	-	0 (0%)
Cortical Ribboning Alone	-	0 (0%)
Data Missing	-	-

Table 35 EEG assessment of unlikely (4.2S) and definitely not (4.3S) RT-QuIC negative cases

	RT-QuIC Positive n=0	**RT-QuIC Negative n=12**
EEG performed		
Yes	-	12/12 (100%)
No	-	0 (0%)
Data missing	-	0 (0%)
EEG Reviewed at NCJDRSU	-	9/12 (75%)
Positive	-	1/9 (11.1%)
Negative	-	8/9 (88.9%)
EEG Reviewed Locally	-	3/12 (25%)
Positive	-	0/3 (0%)
Negative	-	3/3 (100%)

Table 36 Comparison of 14-3-3 and RT-QuIC for unlikely (4.2S) and definitely not (4.3S) cases

	RT-QuIC Positive n= 0	**RT-QuIC Negative n =12**
Median Time to LP from Symptom Onset	-	9.2 (range 0.6-35.2)*
14-3-3		
Positive	-	2/12 (16.7%)
Negative	-	10/12 (83.3%)

Table 37 demonstrates the final diagnosis in the 4.2S and 4.3S sub-cohort.

Table 37 Final diagnosis of the unlikely (4.2S) and definitely not (4.3S) cases

	RT-QuIC Negative n=12
Final Diagnosis	
Neuro-metabolic	1
Neuro-inflammatory	2
Diagnosis Unclear	2
Diagnosis Unclear; patient improved	3
Neurodegenerative- Alzheimer's disease	1
Vascular dementia	1
Mixed dementia	1
Primary psychiatric	1

3.9 Summary of the clinical characteristics and investigations for RT-QuIC positive and RT-QuIC negative cases for all classifications

Table 38 provides an overview of the clinical characteristics and investigation findings for RT-QuIC positive and RT-QuIC negative cases for all classifications. MRI imaging was considered positive only if results were in concordance with the EuroCJD diagnostic criteria (i.e. Basal ganglia signal change +/- cortical ribboning and not isolated cortical ribboning).

	Total N	Mean Age (years)	Median Duration (months)	RCD (%)	Myoclonus (%)	Cerebellar (%)	Visual Symptoms (%)	Mutism (%)	Pyramidal/ Extrapyramidal (%)	MRI Positive (%)	EEG Positive (%)	14-3-3 Positive (%)
1.0												
RT-QuIC +	**35**	**66.6**	**4.47**	**34/35** (97.1%)	**33/34** (97.1%)	**31/35** (88.6%)	**23/32** (71.9%)	**17/33** (51.5%)	**22/32** (68.8%)	**19/31** (61.3%)	**12/33** (36.4%)	**31/35** (88.6%)
RT-QuIC –	**9**	**61.1**	**13.0**	**8/9** (88.9%) -	**7/9** (77.8%)	**7/9** (77.8%)	**6/9** (66.7%)	**3/8** (37.5%)	**3/8** (37.5%)	**5/9** (55.6%)	**2/9** (22.2%)	**5/9** (55.6%)
Data Missing	-	-	-		**1**	**0**	**3**	**3**	**4**	**1**	**2**	**0**
2.0												
RT-QuIC +	**38**	**70**	**4.48**	**38/38** (100%)	**37/38** (97.4%)	**32/32** (100%)	**26/38** (68.4%)	**21/36** (80.8%)	**29/36** (80.6%)	**24/38** (63.2%)	**11/38** (28.9%)	**34/38** (89.5%)
RT-QuIC –	**7**	**66.9**	**3.57**	**7/7** (100%)	**7/7** (100%)	**5/7** (71.4%)	**3/7** (42.9%)	**2/6** (33.3%)	**4/6** (66.7%)	**3/7** (42.9%)	**3/7** (42.9%)	**6/7** (85.7%)
Data Missing	-	-	-	**0**	**0**	**6**	**0**	**3**	**3**	**0**	**0**	**0**
3.0												
RT-QuIC +	**3**	**67**	**4.26**	**3/3** (100%)	**3/3** (100%)	**3/3** (100%)	**1/3** (33.3%)	**0/3** (0%)	**1/3** (33.3%)	**0/3** (0%)	**0/2** (0%)	**0/3** (0%)
RT-QuIC –	**2**	**64**	*	**2/2** (100%)	**1/2** (50%)	**2/2** (100%)	**1/2** (50%)	**0/3** (0%)	**1/2** (50%)	**0/2** (0%)	**0/2** (0%)	**0/2** (0%)
4.1												
RT-QuIC +	**2**	**70**	**9.1**	**2/2** (100%)	**0/2** (0%)	**0/2** (0%)	**1/2** (50%)	**0/2** (0%)	**1/2** (50%)	**1/2** (50%)	**0/2** (0%)	**0/2** (0%)
RT-QuIC –	**4**	**58.5**	**29.5**	**2/4** (50%)	**1/4** (25%)	**3/4** (75%)	**1/4** (25%)	**1/4** (25%)	**1/4** (25%)	**2/4** (50%)	**0/4** (0%)	**0/4** (0%)
4.2/4.3												
RT-QuIC -	**12**	**72**	**9.1**	**9/12** (75%)	**7/12** (58.3%)	**4/12** (33.3%)	**6/12** (50%)	**2/12** (16.7%)	**6/12** (50%)	**0/10** (0%)	**1/12** (8.3%)	**2/12** (16.7%)

Table 38 Summary of the clinical characteristics and investigations for RT-QuIC positive and negative cases for all classifications

3.10 Review of the operational parameters of RT-QuIC and influence on result

3.10.1 Influence of timing of lumbar puncture on the RT-QuIC result

Figures 11 and 12 demonstrate the influence of the timing of the LP on the RT-QuIC result for each classification.

Figure 11 Effect of Timing of LP on a Positive RT-QuIC Result

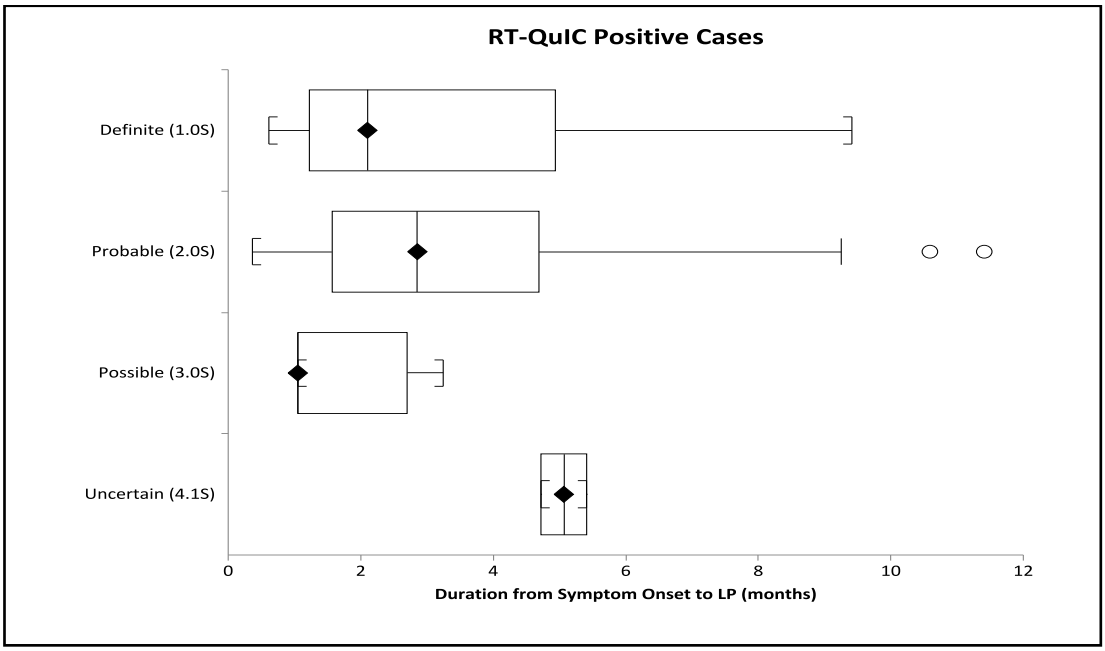
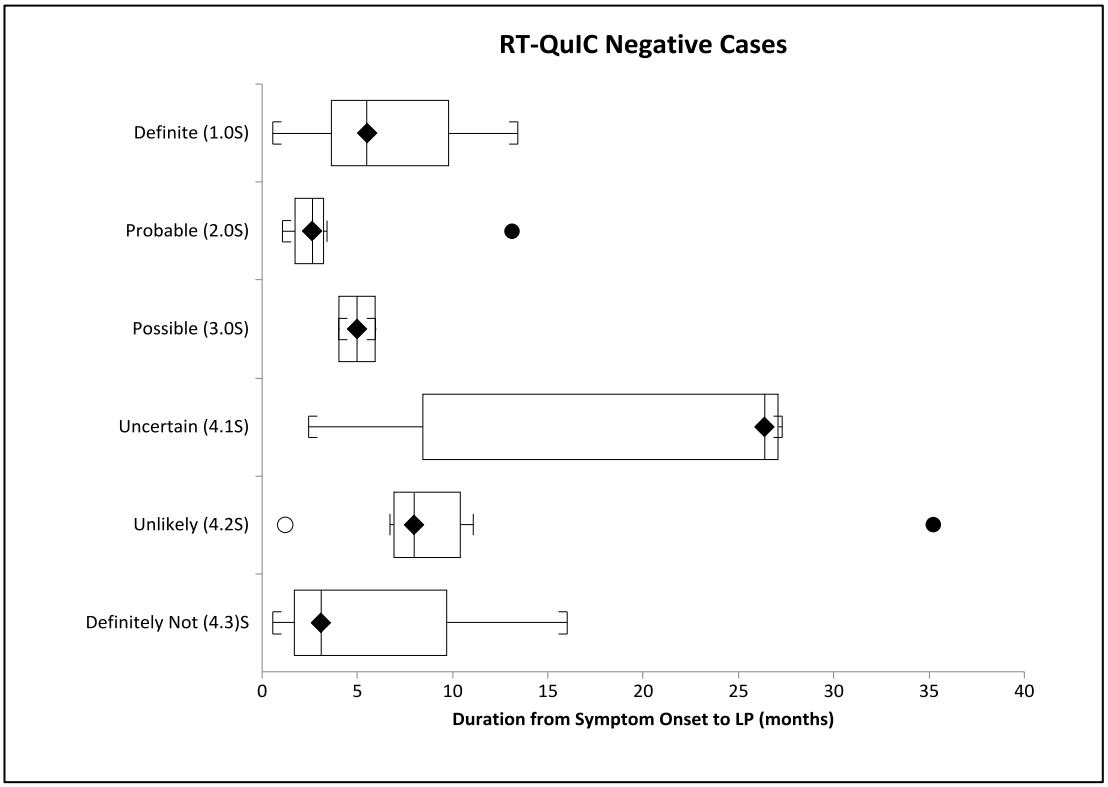


Figure 12 Effect of Timing of LP on a Negative RT-QuIC Result



3.10.2 Influence of CSF volume on RT-QuIC Result

During the initial data period, 15µl of CSF was used although over the course of the study, it was found that 30µl gave better discrimination between positive and negative RT-QuIC results. However, some CSF samples from cases of suspected sporadic CJD had a negative RT-QuIC response at 30µl but a postive RT-QuIC response was obtained using 15µl. Therefore, CSF samples were tested at both 15µl and 30µl.

3.10.3 Timing of RT-QuIC result in comparison to other investigations

Below are a series of figures illustrating the timing of the LP , EEG and MRI results for 1.0S and 2.0S cases. This is followed by Table 4.35 which provides a summary of the timing of all of the investigations for each classification.

Figure 13 Definite Cases (1.0S) Time from LP to RT-QuIC Result

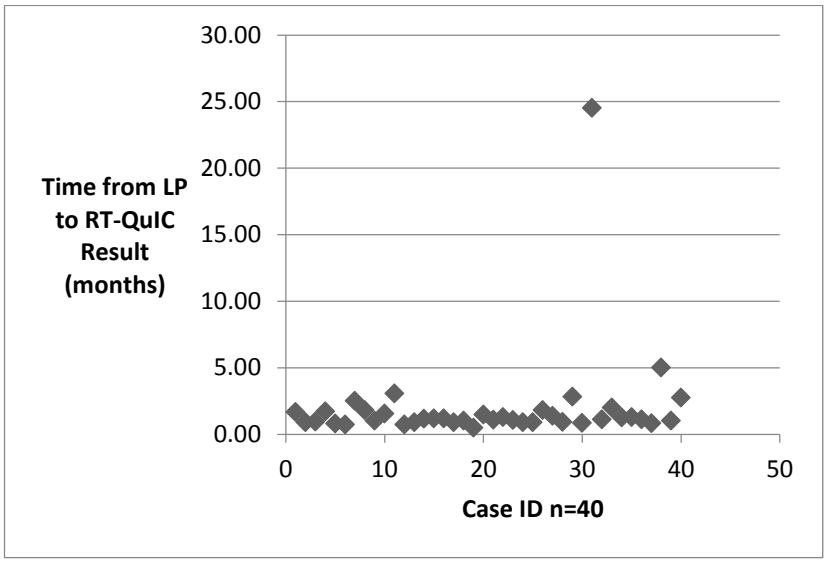


Figure 14 Definite Cases (1.0S) Time from LP to 14-3-3 Result

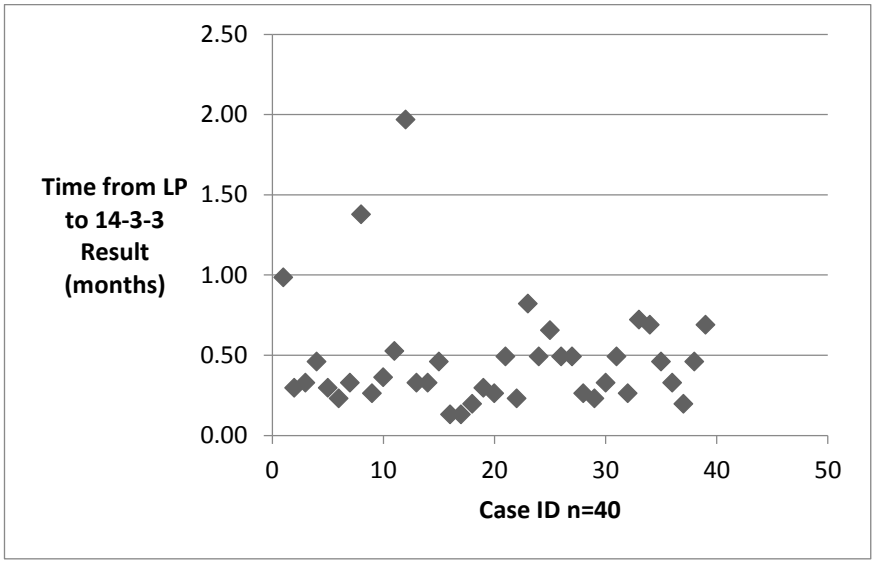


Figure 15 Definite Cases (1.0S) Time from Symptom Onset to RT-QuIC Result

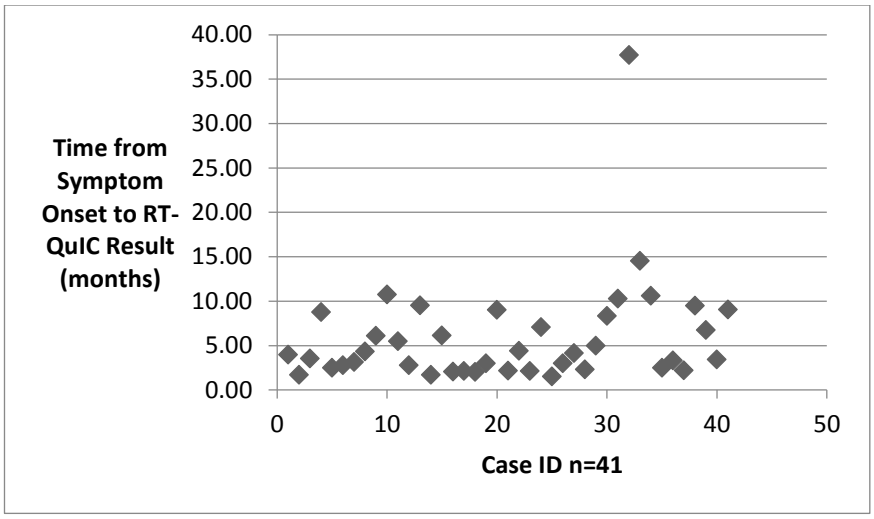


Figure 16 Definite Cases (1.0S) Time from Symptom Onset to 14-3-3 Result

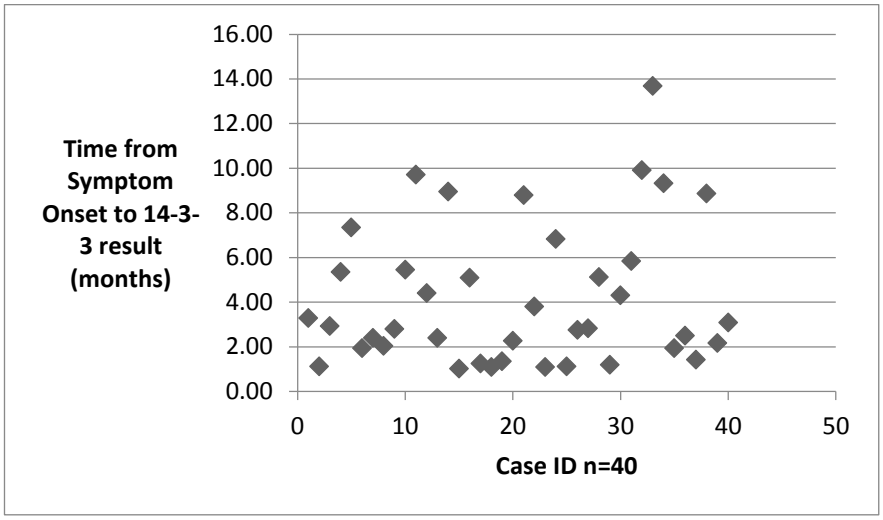


Figure 17 Definite Cases (1.0S) Time from Symptom Onset to a Positive EEG Result

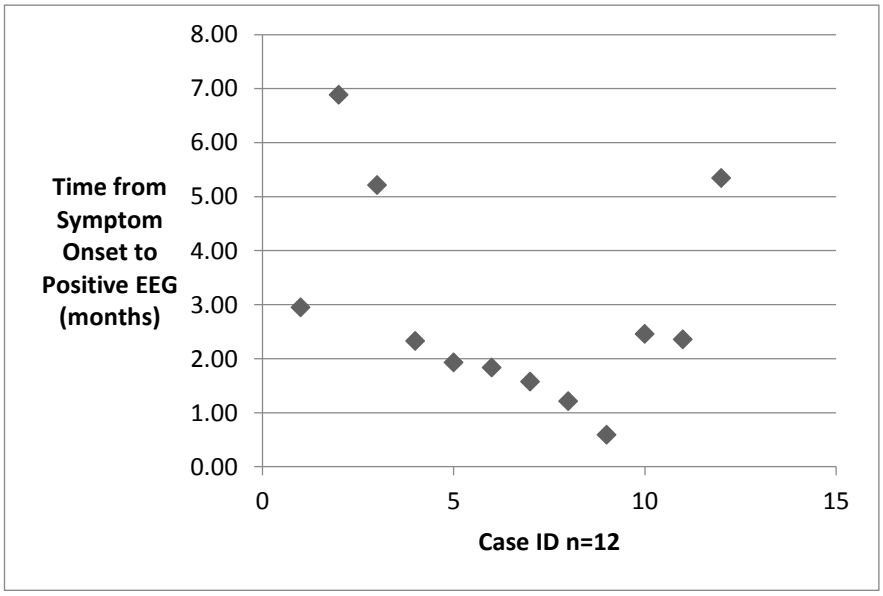


Figure 18 Definite Cases (1.0S) Time from Symptom Onset to a Positive MRI Result

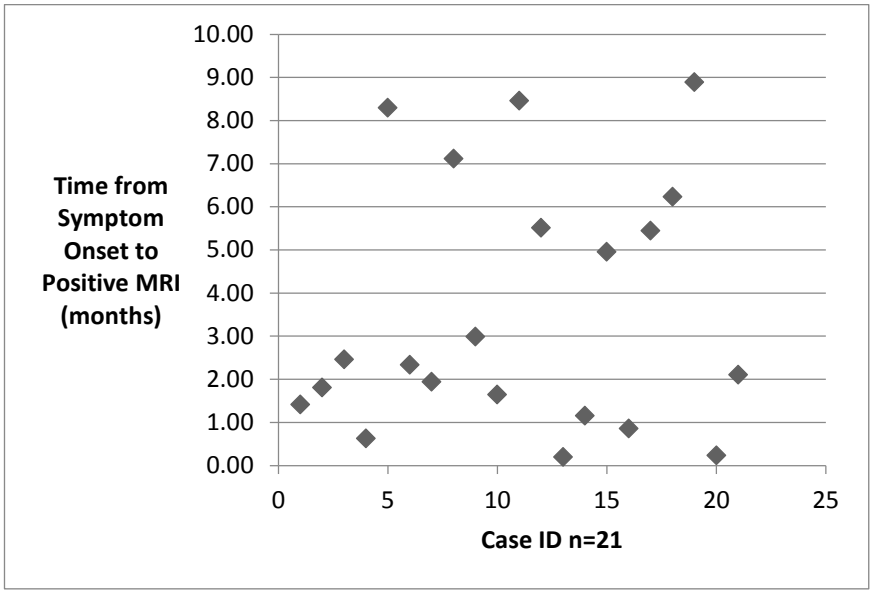


Figure 19 Probable Cases (2.0S) Time from LP to RT-QuIC Result

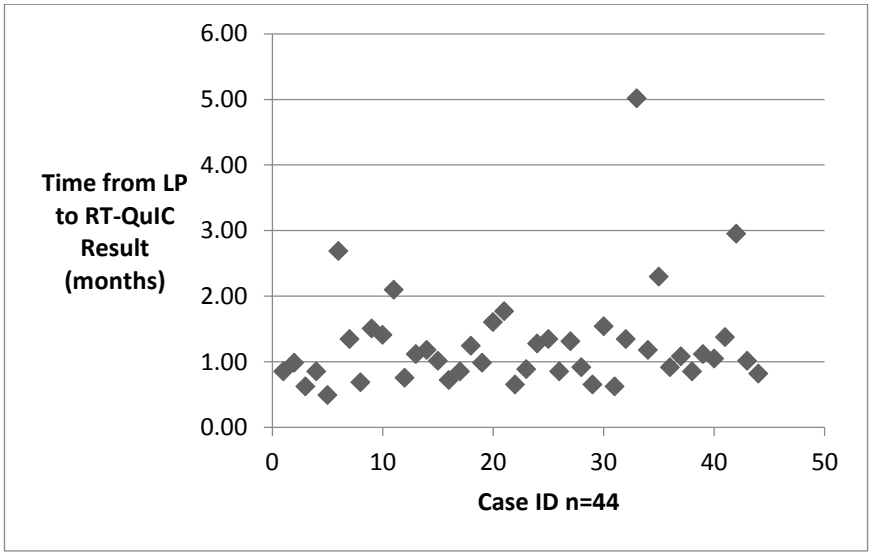


Figure 20 Probable Cases (2.0S) Time from LP to 14-3-3 Result

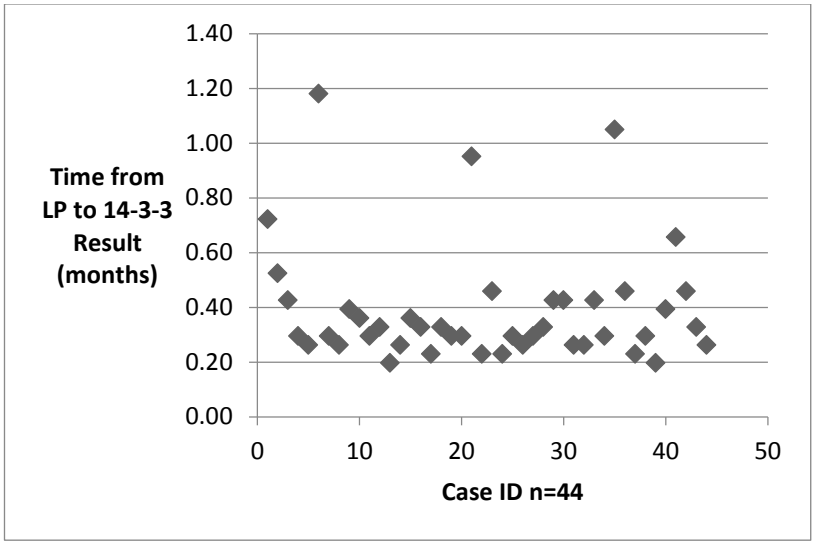


Figure 21 Probable Cases (2.0S) Time from Symptom Onset to RT-QuIC Result

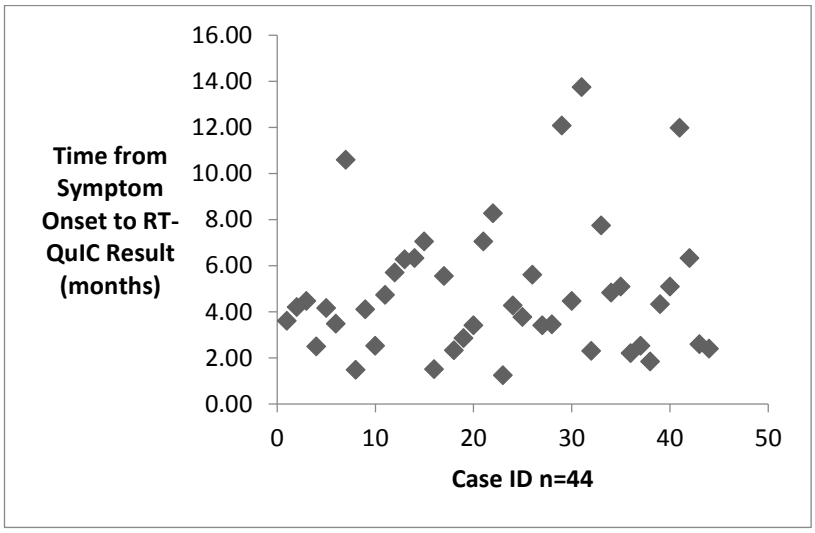


Figure 22 Probable Cases (2.0S) Time from Symptom Onset to 14-3-3 Result

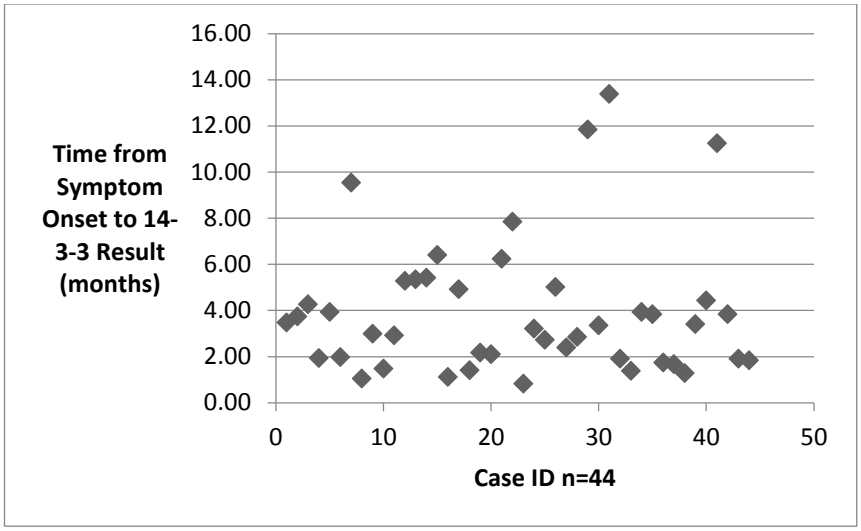


Figure 23 Probable Cases (2.0S) Time from Symptom Onset to a Positive EEG

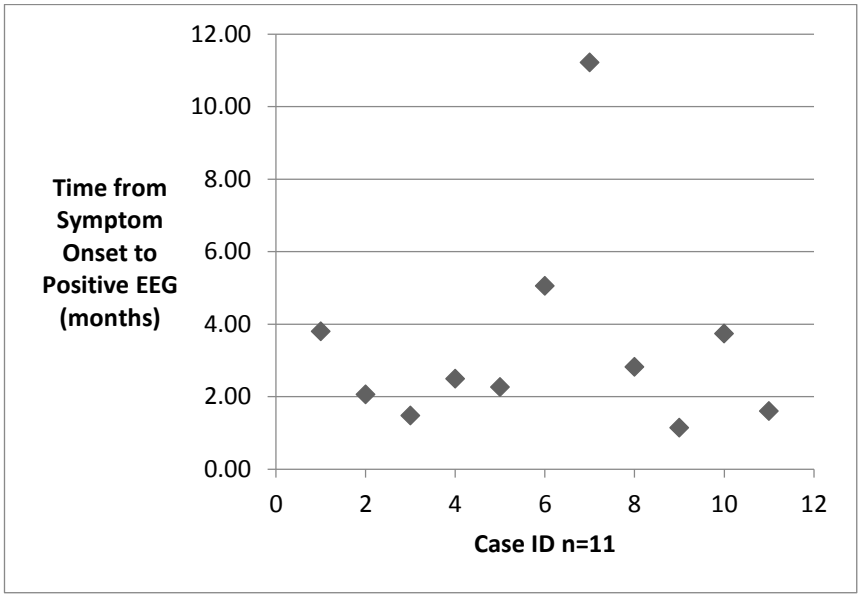
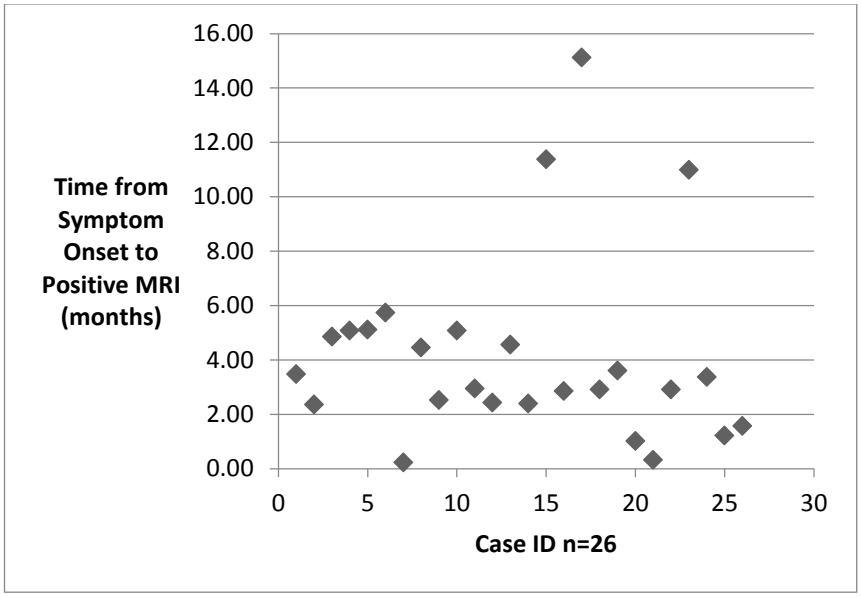


Figure 24 Probable Cases (2.0S) Time from Symptom Onset to a Positive MRI Result



	Total Number	Time from Symptom Onset to Positive MRI (Range in months)	Time from Symptom Onset to Positive EEG (Range in months)	Time from Symptom Onset to 14-3-3 Result (Range in months)	Time from Symptom Onset to RT-QuIC Result (Range in months)	Time from LP to 14-3-3 Result (Range in months)	Time from LP to RT-QuIC Result (Range in months)
1.0S	**44**	**24/44 (54.5%)** **3.6** (0.2-8.9)	**14/42 (33.3%)** **2.9** (0.6-6.9)	**40/44 (90.9%)** **4.2** (1-13.7)	**41/44 (93.2%)** **5.9** (1.5-37.7)	**40/44 (90.9%)** **0.5** (0.1-2)	**40/44 (90.9%)** **1.4** (0.5-5)
2.0S	**45**	**27/45 (60%)** **4.2** (0.2-15.1)	**11/45 (24.4%)** **3.4** (1.2-11.2)	**44/45 (97.8%)** **3.9** (0.8-13.4)	**44/45 (97.8%)** **4.9** (1.3-13.7)	**44/45 (97.8%)** **0.4** (0.2-1.2)	**44/45 (97.8%)** **1.3** (0.5-5)
3.0S	**5**	**None positive**	**None positive**	**4/5 (80%)** **4.5** (1.5-6.1)	**5/5 (100%)** **5** (2.6-6.5)	**4/5 (80%)** **0.4** (0.1-0.7)	**5/5 (100%)** **1.1** (0.5-1.7)
4.1S	**6**	**3/6 (50%)** **11.1** (2.3-26.3)	**None Positive**	**6/6 (100%)** **12.4** (2.7-27.6)	**6/6 (100%)** **12.8** (3.3-28.2)	**5/6 (83.3%)** **0.4** (0.2-0.5)	**5/6 (83.3%)** **0.8** (0.6-0.9)
4.2/4.3S	**12**	**None Positive**	**1/12 (8.3%)** **2.8** (1 positive)	**12/12 (100%)** **9.4** (1.2-35.5)	**12/12 (100%)** **9.8** (2.3-35.8)	**11/12 (83.3%)** **0.53** (0.2-1.2)	**11/12 (91.7%)** **1** (0.4-1.8)

Table 39 Summary of timing of investigations for all classifications

3.10.3.1 Definite sporadic (1.0S) Group

The time from symptom onset to RT-QuIC result could not be calculated in 3 cases as the date of the RT-QuIC result was missing. The time taken from LP to RT-QuIC result was prolonged at 24.5 months in one case. This case was referred for CSF and formal referral out with the data period but reviewed during the data period and the RT-QuIC performed retrospectively. This case was therefore not included in the median duration to result. The time between symptom onset to 14-3-3 result could not be calculated in 4 cases as the date of the 14-3-3 result was missing. 42/44 cases had an EEG performed and data was missing for the remaining 2 cases. 14 were positive. The time from symptom onset to a positive EEG result could be calculated in 12/14. 24/44 cases had a positive MRI of which the duration from symptom onset to a positive MRI result could be calculated in 21/24. The remaining 3 cases could not be calculated as the date of the MRI result was missing.

3.10.3.2 Probable Sporadic (2.0S) Group

The time from symptom onset to the RT-QuIC result could not be calculated in one case as the date of the RT-QuIC result was missing. The time taken from the LP to the 14-3-3 result could not be calculated in one case as the date of the 14-3-3 result was missing. The duration from symptom onset to RT-QuIC result could not be calculated in one case as the date of the RT-QuIC result was missing. Duration between symptom onset and 14-3-3 result could not be calculated in one case as the date of the 14-3-3 was missing. EEG was performed in 42/45 cases and was positive in 14/42. The time to positive result could not be calculated in 3 cases as the date of the positive EEG was missing. 27/45 cases had a positive MRI. Data was missing in one case. The time to a positive result could not be calculated in one case as the date of the MRI result was missing.

3.10.3.3 Possible sporadic (3.0S) Group

The time from LP to 14-3-3 result could not be calculated one case as the 14-3-3 result date was missing. Time from symptom onset to 14-3-3 result could not be calculated in one case for the same reason. All 5 cases underwent a MRI, none of which were positive. 4/5 cases had an EEG, none of which were positive.

3.10.3.4 Diagnosis uncertain (4.1S) Group

The time from LP to the RT-QuIC result was calculated in 5/6 cases. The date of LP was missing in one case. The time from LP to 14-3-3 result was calculated in 5/6 due to one case with a missing date of LP. All 6 underwent an EEG, none of which were positive. All 6 underwent a MRI with half of these positive.

3.10.3.5 Unlikely sporadic or definitely not sporadic (4.2S/4.3S) Group

The time from LP to RT-QuIC result was calculated in 11/12. The date of the LP was missing in one case. This was similar for the time from the LP to 14-3-3 result. All 12 underwent an EEG with only one case reported as positive. 10/12 had a MRI, none of which were positive.

3.11 Overall Sensitivity and Specificity of RT-QuIC

The following tables illustrate the investigation results for each classification and also the specificity, sensitivity and positive predictive value of each test.

Table 40 Investigations results by classification

Classification (EuroCJD Criteria)	Total	RT-QuIC positive	14-3-3 Positive	EEG Positive	MRI Positive (Basal ganglia change)	MRI Positive (Cortical ribboning only)
Definite	44	35 (80%)	36 (82%)	14 (32%)	24 (55%)	6 (14%)
Probable	45	38 (84%)	40 (89%)	14 (31%)	27 (60%)	4 (9%)
Possible	5	3 (60%)	0 (0%)	0 (0%)	0 (0%)	3 (60%)
Uncertain	7	2 (29%)	2 (29%)	0 (0%)	3 (43%)	2 (29%)
Non Case Clinically	7	0 (0%)	2 (29%)	0 (0%)	0 (0%)	0 (0%)
Non Case Pathologically	4	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)

Table 41 Sensitivity, specificity and positive predictive value of all tests

Definite Sporadic CJD	RT-QuIC	14-3-3	EEG	MRI (Basal Ganglia change)	MRI (Cortical ribboning only)
Sensitivity	80%	82%	32%	55%	14%
Specificity	100%	82%	91%	100%	
Positive Predictive Value	100%	95%	93%	100%	

3.12 Case Vignettes

Below are a series of case vignettes where the RT-QuIC result was particularly useful. These are divided into RT-QuIC negative and RT-QuIC positive cases.

3.12.1 Cases where RT-QuIC was Positive

3.12.1.1 Case 1

A 47 year old male patient presented with a 1 year history of prominent neuropsychiatric features and painful limbs. Initial MRI brain and CSF 14-3-3 were not supportive although the RT-QuIC test was positive. The clinical course progressed to a clinical phenotype supportive of sCJD and repeat MRI brain and 14-3-3 later became positive. Post mortem and codon 129 genotyping was not performed and the final classification based on the WHO criteria was a Probable case (2.0S).

3.12.1.2 Case 2

A 75 year old female presented with a neurological syndrome of rapidly progressive dementia and progressed to develop cerebellar signs and akinetic mutism. However, the MRI brain and EEG were not supportive. CSF 14-3-3 and RT-QuIC were both positive. However, the clinical picture was complicated by a possible malignant lesion of the pancreas identified on CT imaging suggesting the possibility of a paraneoplastic process. In addition, a significantly raised anti-thyroid peroxidase antibody was found suggesting the possibility of Hashimoto's thyroiditis. This patient underwent a course of steroid treatment although continued to progress and died. The final WHO diagnostic classification during life was Uncertain (4.1S) due

to the fact that an alternative cause could not be fully excluded. Post mortem examination confirmed sCJD and the classification was upgraded to a definite (1.0S) case. Genotyping and PrP^{Sc} type was not available.

3.12.1.3 Case 3

A 77 year old female presented with bilateral deafness and later developed rapidly progressive dementia, pyramidal signs and myoclonus. MRI, EEG and 14-3-3 were not supportive and the highest classification during life was a Possible case (3.0S). Post mortem examination confirmed sCJD and this case was re-classified as definite (1.0S). Data on genotype and PrP^{Sc} type was not performed. The RT-QuIC test was positive in this case.

3.12.2 Cases where RT-QuIC was negative

3.12.2.1 Case 1

A 73 year old male presented with weight loss and insomnia followed by rapidly progressive dementia, ataxia, myoclonus and visual symptoms. MRI and EEG were not supportive of sCJD but the 14-3-3 test was positive. It was the impression of the local team that this was a case of sCJD. Based on the ongoing progression of the patient's condition and following the WHO diagnostic criteria, this case was initially classified as 2.0S. However, at alter date the anti-voltage gated potassium channel antibody was returned as significantly positive confirming the diagnosis of autoimmune encephalitis. The patient is still alive and the case was re-classified as 4.2S. RT-QuIC was negative in this case.

3.12.2.3 Case 2

An 85 year old male presented with rapid cognitive decline and progressed to akinetic mutism and myoclonus. The case was complicated by subdural haematomas and subsequent seizure activity. The 14-3-3 and MRI were not supportive of sCJD but the EEG was positive and overall this was thought to be a case of sCJD. However, post mortem examination demonstrated changes consistent with Alzhemier's disease and not sCJD. The RT-QuIC result was negative in this case.

3.13 Diagnostic outcome for cases that were referred for CSF only

As detailed in the methodology section, the NCJDRSU receives a large proportion of CSF for analysis of 14-3-3 in whom CJD is being considered a possibility although not yet regarded as a suspected case for formal referral. The clinical details provided by the local clinicians at initial referral were reviewed along with the 14-3-3 and RT-QuIC results for all of the non-referred cases within the data period. All local clinicians were written to requesting the final diagnosis. Within the data period, 221 samples of CSF were sent to the NCJDRSU requesting analysis for 14-3-3 for patients in whom CJD was being considered a possibility. 122 responses were received from the local clinicians detailing the overall progress of the patient and diagnosis where possible. These are shown in table 41. 5 cases were categorised as 'other'. Diagnoses included: nephrogenic diabetes insipidus and an extrapyramidal syndrome secondary to long term valproate use; resection of an ependymoma complicated by hydrocephalus and ventriculitis; post-flu immunisation encephalopathy and segmental dystonia.

Three cases of CJD were identified. One case was an iatrogenic case of CJD confirmed by post mortem examination. One case was labelled as sporadic CJD on the death certificate but this was not confirmed by post mortem and was not formally referred. This case was RT-QuIC negative. The final case was RT-QuIC positive and was confirmed at post mortem. This patient was not formally referred to the NCJDRSU for review.

Table 43 Final outcome for non-referred cases in whom CJD considered a possibility

Diagnosis/Overall Outcome	Case Number n=122 (%)
Dementia	
Vascular	**5 (4.1%)**
Frontotemporal	**9 (7.4%)**
Corticobasal Degeneration	**2 (1.6%)**
Parkinson's Disease with Dementia	**1 (0.8%)**
Lewy Body Dementia	**9 (7.4%)**
Alzheimer's Disease	**13 (10.7%)**
Mixed Dementia	**5 (4.1%)**
Unclassified	**2 (1.6%)**
Inflammatory/Autoimmune	**13 (10.7%)**
Infection	**7 (5.7%)**
Metabolic/Alcohol related	**7 (5.7%)**
Diagnosis Unclear- Not CJD	**20 (16.4%)**
Malignant/Paraneoplastic	**4 (3.3%)**
Motor Neuron Disease	**3 (2.5%)**
Huntington's Disease	**2 (1.6%)**
Progressive Supranuclear Palsy	**1 (0.8%)**
Vascular/Stroke	**3 (2.5%)**
Psychiatric/Functional	**8 (6.6%)**
Other	**5 (4.1%)**
CJD	
PM confirmed Sporadic	**1 (0.8%)**
Non PM Confirmed Sporadic	**1 (0.8%)**
PM confirmed iatrogenic CJD	**1 (0.8%)**

Chapter 4

Discussion

4 Discussion

4.1 Structure of Discussion

Human prion diseases are highly heterogenous and invariably fatal neurological disorders. The commonest form is sporadic CJD which accounts for approximately 85% of cases[103]. The exact infectious mechanism remains unknown although the most widely accepted theory is the protein hypothesis where a normal cellular protein, PrP^{C} undergoes post conformational misfolding thus resulting in the disease associated form of the protein, PrP^{Sc} [2, 4]. This abnormal prion protein subsequently aggregates to form amyloid fibrils and although the pathological mechanism is not yet fully understood, the recognised pathological hallmarks of the disease which includes astrocytosis, gliosis and neuronal cell loss[5]. The clinical heterogeneity of sCJD results from the influence of the host PrP genotype and the conformational pattern of the pathological PrP^{Sc} as described[22]. The variation in clinical and pathological phenotype has led to difficulties with making an early and accurate diagnosis[24, 25]. Although sCJD is not infectious per se, it is transmissible and concern has been raised about subclinical cases who remain asymptomatic although may be infectious and therefore pose a risk from a public health perspective[27]. Preventative measures have been useful in dramatically reducing the incidence of iatrogenic transmission of variant CJD although these measures do not always apply to sporadic CJD which arises spontaneously. In addition, an early diagnosis alleviates family distress and will confirm or refute the diagnosis thus allowing a potentially treatable condition to be diagnosed earlier. Confirming sCJD at an early stage provides greater opportunity to identify potential therapeutic treatments.

The development and revision of diagnostic criteria over the years has proven to be very useful in diagnosing sCJD. The EuroCJD criteria is based on a recognisable clinical presentation combined with diagnostic tests that include EEG, MRI and CSF biomarkers including 14-3-3[165]. However, diagnostic criteria was mainly developed for epidemiological purposes and does not take into account the clinical

heterogeneity of the condition which poses as a major diagnostic challenge in differentiating prion diseases from other neurological disorders[178]. CJD is increasingly recognised as in the differential diagnosis of rapidly progressive neurological syndromes, highlighting the need for reliable tools to make an early and accurate diagnosis. The ageing population, improved awareness of the clinical heterogeneity of sCJD and the identification of potentially treatable disorders that manifest as rapidly progressive neurological syndromes have all contributed to this problem.

Currently at least 6 major subtypes of sCJD are recognised based on the *PRNP* codon 129 genotype and PrP^{Sc} Type. The number and variety of clinical symptoms, rate of disease progression and underlying brain pathology vary significantly amongst these individual subtypes and therefore challenge the accuracy and of the diagnostic criteria[22, 24, 25,178]. However, the definitive means of diagnosis remains neuropathological confirmation of brain tissue obtained either by post mortem or brain biopsy[165]. The latter is invasive with many patients considered to be unfit for the procedure due to the nature of the condition as well as the iatrogenic risk to others from the operation[173]. The diagnostic accuracy of the criteria has been challenged particularly in relation to potentially missing cases that present atypically where early symptoms can be non-specific and conventional tests may be normal[174-184].

This identified the need for a more accurate and robust test that is based on the disease process itself. A number of bioassays have been developed with the most recent named RT-QuIC[196-204]. This test exploits the seeding process of the abnormal prion protein and has been demonstrated in preliminary studies to diagnose sCJD with a high degree of sensitivity and specificity[194, 195]. This study aimed to assess the diagnostic accuracy of RT-QuIC via a prospective study over an 18 month period by evaluating the sensitivity and specificity. In addition, it aimed to look at the utility of the test in comparison to more conventional techniques as stipulated by the EUROCJD criteria and assess the ability of this novel test to identify cases that may have been missed by conventional diagnostic techniques. These cases will be examined in detail. The influence of age of onset, disease duration, codon 129 and

timing of lumber puncture will also be discussed and their influence on the outcome of RT-QuIC in comparison to current literature.

During the course of this study, certain operational parameters and limitations of the test in our centre have been reported and will be discussed. Since the onset of this study, further studies have been carried out in attempt to improve the accuracy of the test. There have also been large multicentre studies that have improved the standardisation, validation and overall reproducibility and accuracy of the test.

Finally, the future of RT-QuIC in the diagnoses of other neurodegenerative disorders where the molecular mechanism of disease is thought to be based on misfolding of a host protein in to a disease associated form will be considered as part of the role of RT-QuIC in future research.

4.2 Sensitivity and Specificity of RT-QuIC

This study has demonstrated that RT-QuIC is a robust test with a high degree of sensitivity and specificity which is in concurrence with other studies. Several research groups have now conducted large studies in which CSF samples collected from sCJD cases and controls were evaluated using RT-QuIC assays[24]. Collectively, these studies provide hundreds of sCJD cases and an even larger number of controls. Diagnostic sensitivities observed range from 77-97% and specificities 99-100%[195, 203-208, 212].

It has been demonstrated that there is no apparent correlation between the RT-QuIC response obtained in confirmed sCJD cases and any of the clinical features such as age at onset of the disease, disease duration or timing of the lumber puncture. The lack of influence timing of lumber puncture has on the test suggests that CSF samples tested early in the disease course are unlikely to yield false positive results[203, 212].

A large number of non-CJD CSF samples have been analysed in these studies and only two have returned a positive RT-QuIC result[212]. One of these cases had a working diagnosis of frontotemporal dementia and was lost to follow up. The second case was given a diagnosis of a paraneoplastic syndrome although it remains unclear as to whether this was confirmed at autopsy. Overall, the specificity of RT-QuIC is approaching 100% and has been demonstrated to be significantly better than other surrogate markers such as 14-3-3[212].

However, RT-QuIC does not have perfect sensitivity and fails to identify 10-20% of cases of sCJD. PrP^{Sc} accumulates in the olfactory epithelium in patients with sporadic CJD which suggests that evaluation of these tissues may be useful in providing another strategy for antemortem diagnosis of sCJD[24, 208]. One study has

shown that the use of RT-QuIC using nasal brushings has the ability to provide a diagnosis with a high degree of sensitivity and specificity[208]. Samples of the olfactory epithelium can be collected by a simple and gentle brushing technique although does involve directly visualising the nasal vault with a rigid, sheathed fibroscope. Three-four brushings per nostril are obtained to maximise the liklihood of contacting the olfactory mucosa as adjacent respiratory mucosa is low in PrP^{Sc}. To date, nasal brushings from a total of 43 patients and a total of 43 non CJD controls were assessed and indicated an overall diagnostic sensitivity of 97.5% and specificity of 100%[208]. This sensitivity was superior to that achieved using CSF samples from the same patients. It was suggested that olfactory mucosa specimens provide a significantly stronger RT-QuIC response than CSF samples obtained from the same patient with an overall higher sensitivity than CSF. However further validation via this means of testing is still required to establish the overall diagnostic performance of the test especially with regard to how early in the course of the disease the infectivity can be detected[208].

In a more recent study, the diagnostic sensitivity of RT-QuIC was assessed using CSF and olfactory mucosa from 86 patients classified as probable, possible or suspected sCJD. The CSF was tested using standard and improved RT-QuIC conditions whereas the olfactory mucosa was tested using standard conditions only. Sensitivity in the CSF samples was 95% and olfactory mucosa 97% and 100% specific. However, if both samples were tested together then this is improved to an overall sensitivity and specificity of 100%. This led to the recommendation that both CSF and olfactory mucosa should be tested for RT-QuIC to optimise diagnostic accuracy[213].

Despite these encouraging findings, olfactory epithelial testing is invasive and is not part of the routine diagnostic work up for a patient suffering from a rapidly progressive dementia. In addition, the stronger result suggests possible infectivity and therefore may have biosafety implications[24, 208]. Therefore CSF remains a

primary diagnostic sample for RT-QuIC sampling. However, if the CSF RT-QuIC is negative or a lumbar puncture has not been feasible and there is still a high degree of suspicion of that sCJD is the diagnosis then testing olfactory mucosa may be a feasible option if this test undergoes adequate validation and standardisation[24].

This study has shown that RT-QuIC has a high degree of sensitivity and specificity in line with other studies. Since the onset of this current study, two international studies have demonstrated that this is a robust and reproducible test amongst laboratories in different countries using a range of recombinant PrP^{C} sources and instrumentation produced identical results when analysing the same set of CSF samples[205, 214]. The WHO have recognised the usefulness of this test and now included it into the diagnostic criteria as of January 2017 which has now replaced the diagnostic criteria that this study was based on and is detailed below[113]:

Diagnostic criteria for surveillance of sporadic CJD from 1 January 2017

1.1 **DEFINITE:**

Progressive neurological syndrome **AND**

Neuropathologically **or** immunohistochemically **or** biochemically confirmed

1.2 **PROBABLE:**

1.2.1 I + two of II and typical EEG*

OR 1.2.2 I + two of II and typical MRI brain scan**

OR 1.2.3 I + two of II and positive CSF 14-3-3

OR 1.2.4 Progressive neurological syndrome and positive RT-QuIC in CSF or other tissues

1.3 **POSSIBLE:**

I + two of II + duration < 2 years

I Rapidly progressive cognitive impairment

II
- A Myoclonus
- B Visual or cerebellar problems
- C Pyramidal or extrapyramidal features
- D Akinetic mutism

*Generalized periodic complexes
**High signal in caudate/putamen on MRI brain scan or at least two cortical regions (temporal, parietal, occipital) either on DWI or FLAIR

4.3 Definite and Probable Cases

A total of 115 cases formally referred to the NCJDRSU during the study period underwent CSF testing for both RT-QuIC and 14-3-3. 44 of these were classified as definite cases according to the EUROCJD diagnostic criteria 2009. 35 cases were RT-QuIC positive and 9 were negative. There was no significant difference between age at onset between the positive and negative groups. However the negative group had a longer median disease duration of 13 months (range 2.2-32.4) and longer disease duration was significantly more likely to produce a negative RT-QuIC result. The majority of both groups presented with a rapidly progressive dementia process although the RT-QuIC negative group were more likely to present with an 'Other' mode of presentation. In comparison, 45 cases were classified as probable of which 38 were positive for RT-QuIC and 7 were negative. There was no observed difference in terms of age or disease duration between these groups and no significant influence of either of these factors on the outcome of the RT-QuIC result. However, the positive group were more likely to present with a rapidly progressive dementia, 50% and 28.5% respectively. The RT-QuIC negative group were more likely to present with a psychiatric presentation or stroke-like presentation, 28.5% and 14.2% respectively.

4.3.1 Influence of Codon 129 and PrP^{Sc} Type

As previously discussed, patients with sporadic CJD can be characterised according to the *PRNP* codon 129 genotype and the biochemical characteristics of PrP^{Sc} into 6 molecular subtypes[22]. The subtypes MM1 and MV1 represent the classic clinical phenotype and subtypes MM2 and VV1 are less typical[22].

In the definite group, the RT-QuIC positive cases were more likely to have a MM codon 129 genotype than the negative group, 58% and 33.3% respectively. The negative cases were more likely to be VV genotype compared to the RT-QuIC positive group, 44.4% compared to 22.5% respectively. These findings did not reach statistical significance. In the instances where it was possible to fully classify the definite cases by codon 129 genotype and PrP^{Sc} type (as described by Parchi et al), the majority of the RT-QuiC positive group were of a MM1 subtype. The RT-QuIC negative group were more likely to be of the less common subtypes including VV1, MM2A, MV2A and MV mixed. None of the RT-QuIC negative group had a MM1 subtype. Due to lack of post mortem in the probable group, PrP^{Sc} cannot be assessed. In terms of codon 129 genotype, 17/38 (44.7%) of the positive RT-QuIC group underwent genotyping. 9 (52.9%) were MM, 1 (5.9% was MV and 7 (41.2%) was VV. In the RT-QuIC negative group, 2/7 (28.6%) underwent genotyping. 1 (50%) was MM and 1 (50%) was VV.

The numbers in this study are too small to draw any strong conclusions from, however there have been recent larger studies that have investigated the relationship between codon 129 genotype and PrP^{Sc} Type on the outcome of the RT-QuIC response and overall result as detailed below.

Peden et al (2012)- reported that rapid and early seeding was seen with the most commonly occurring phenotypes of sCJD (MM1/MV1, VV2 and MV2). In addition, Atarashi et al also reported (2011) that human recombinant PrP converted equally well in the presence of of sCJD MM1 and MM2 brain seeds. Peden et al (2012) did however find lower seeding efficiency and less reproducible responses when using MM2C and VV1 brain samples[221].

Cramm et al (2015) conducted a CSF study to determine the effects of codon 129, prion disease type and PrP^{Sc} type and other disease related factors on the RT-QuIC response. The effect of codon 129 genotype or PrP^{Sc} type in CSF was assessed separately by performing a comparative analysis of three sCJD genotypes (MM, MV, VV) and two PrP^{Sc} types (1 and 2). By using this approach, they assessed both variables separately and assessed the impact of codon 129 genotype on the patient groups of the same PrP^{Sc} type. Similarly, sCJD cases with the same codon 129 genotype but different PrP^{Sc} type were examined. Results indicated that neither the PrP^{Sc} type or the codon 129 genotype alone had a significant impact on the RT-QuIC response. However, it was found that codon 129 genotype within Type 1 patients was associated with a faster result in MM patients than MV and VV. This effect was not observed in Type 2 candidates. When analysing subgroups of patients the other way, patients exhibiting the same genotype but different PrP^{Sc} type (i.e. MM1 vs MM2, MV1 vs MV2), no difference was observed. In terms of what other factors could influence the kinetics of the RT-QuIC response, this study also reported that disease duration had an impact on the seeding efficiency of PrP^{C}. For example, those with a shorter disease course showed higher seeding efficiency in the RT-QuIC response. As demonstrated in this study, the disease duration in the definite RT-QuIC negative group was more prolonged and this may be relevant to the seeding efficiency of RT-QuIC[206].

Foutz et al (2016) reported a recent study assessing the sensitivity and specificity of RT-QuIC with a second generation version of the test on CSF. They performed a

blinded retrospective study and prospective analysis of a cohort of patients suffering from diverse rapidly progressive neurodegenerative disorders but suspected of having prion disease. The results were compared with detailed neuropathological and genetic assessments. The influence of age at onset, disease duration, phenotype and molecular characteristics were assessed. Neuropathological confirmation was performed on 272 cases. The first retrospective cohort was blinded and tested for RT-QuIC and then unblinded and pathology evaluated. It was found that the neuropathologically verified cases that were RT-QuIC positive were associated with an older age, shorter disease course and higher frequency of ataxia. The RT-QuIC was more frequently negative in the MM2 and no correlation was observed between the RT-QuIC result and codon 129 polymorphism alone, gender, EEG or MRI. It was also found that RT-QuIC was superior to 14-3-3[25].

The overall specificity was 98.5%. The reason it was not 100% was due to 1 case which repeatedly tested positive to CSF RT-QuIC but neuropathology demonstrated lewy body dementia. However, on further examination of the brain homogenate, low levels of PrP^{Sc} were identified. Taking into account the 50 year incubation time of prion diseases and the high sensitivity of RT-QuIC, it is possible that this was a case of dual pathology with the patient in the subclinical phase of the condition but died of an alternative diagnosis. The prospective study revealed a specificity of 100% and sensitivity of 95% with a 100% positive predictive value. CSF 14-3-3 had a much lower specificity of 42.9% and sensitivity of 81% in comparison. Overall the high diagnostic specificity compares well with the results of other studies. The second generation RT-QuIC missed only 5-8% of cases compared to 11-23% reported in the first generation assay.

Lattanzio et al (2017)- recently reported a large study including 1062 patients who presented with a rapid neurological disorder in which CJD was considered a possibility. The number of pathologically confirmed sCJD cases was 186. The overall test sensitivity of RT-QuIC was 82.1% and specificity 99.4% with a PPV of

99%. RT-QuIC sensitivity varied according to the codon 129 genotype and was higher in MM (84.2%) than in MV (72.2%) or VV (79.5%). The specificity was substantially better than 14-3-3. In addition, there was convincing evidence that sensitivity was lowered by atypical subtypes of the disease characterised by PrP^{Sc} Type 2 (VV2, MV2K and MM2C). This variability in seeding activity between subtypes remains unclear. In addition, it remained unclear as to why some neuropathological confirmed cases returned a negative RT-QuIC result. There was no significant correlation between age at onset, disease duration. These results also consolidate the importance of establishing codon 129 genotype in every patient who is being worked up for CJD given the implications it has on clinical phenotype and also prognosis. In addition, it allows for accurate interpretation of investigations that also now include RT-QuIC[212].

4.4 Possible and Uncertain cases

Five cases were classified as possible under the EUROCJD 2009 diagnostic criteria that had both CSF 14-3-3 and RT-QuIC assessed. Of these 3 were positive for RT-QuIC and 2 were negative. Of the RT-QuIC positive group, 1 case presented with rapidly progressive dementia, 1 with cerebellar onset and 1 with an 'Other' presentation (sensory symptoms). Of the negative group, 1 presented with rapidly progressive dementia and 1 with 'Other'. There was no difference in mean age at onset and median disease duration could only be calculated in the positive group (4.26 months). In the negative group, one case was still alive at the time of writing and the other had a disease duration of 11.5 months. All cases underwent codon 129 genotyping. In the positive group, 2 cases were MM and 1 case MV. In the negative group, 1 was MM and the other VV. With regard to investigations, 2 cases in the positive group had a MRI which was reviewed at the NCJDRSU and showed cortical ribboning in isolation which does not support a positive scan by the 2009 diagnostic criteria. The remaining case that was RT-QuIC positive was not sent for review but was considered negative by local services. With regard to the RT-QuIC negative possible cases, 1 case underwent a MRI which showed isolated cortical ribboning. In all possible cases, where EEG and 14-3-3 were performed, these were all negative.

To highlight the usefulness of RT-QuIC in these scenarios, it is of benefit to consider the RT-QuIC positive group on a case by case basis. The first case was a 67 year old patient who presented with a rapidly progressive dementia and later developed other relevant signs such as pyramidal signs, myoclonus and cerebellar signs. The EEG, 14-3-3 were both negative and MRI brain showed cortical ribboning in isolation. The second case was a 68 year old patient with a disease duration of 6.46 months. They presented with sensory symptoms affecting an upper limb and then later developed more typical symptoms including rapidly progressive dementia, myoclonus, visual symptoms and cerebellar signs. MRI revealed isolated cortical

ribboning and 14-3-3 and EEG were both negative. The final case was a 68 year old patient with a disease duration of 4.82 months. This was a cerebellar onset followed by myoclonus and dementia. The MRI was suspicious but not diagnostic of basal ganglia change and both 14-3-3 and the EEG were negative.

By applying the new diagnostic criteria to these possible cases, all of the RT-QuIC positive cases would be classified as probable and one of the negative cases would be classed as probable based on the MRI.

With regard to the cases that were classified as uncertain or 4.1S, 6 underwent CSF analysis for 14-3-3 and RT-QuIC. Two cases were positive for RT-QuIC and 4 negative. The mean age of the negative group was younger (58.5 years) than the positive group (70 years). The duration of illness was also longer in the RT-QuIC negative group with a median disease duration of 29.5 months.

The two RT QuIC positive patients will be discussed separately and in more detail. The first case presented with a rapidly progressive dementia and later developed visual symptoms. MRI brain showed cortical ribboning only and the 14-3-3 was positive but EEG negative. The disease duration was 7.38 months although the patient died without fulfilling the clinical signs required by the WHO diagnostic criteria. However, based on the new WHO diagnostic criteria, this would now be considered a probable case of sporadic CJD and this was considered the most likely diagnosis at the time. The second case was a 73 year old patient who also presented with a rapidly progressive dementia syndrome and went to develop pyramidal signs. CSF 14-3-3 analysis was positive as was the MRI brain but EEG negative. The disease duration was 10.75 months and similar to the last case, the reason this patient did not fulfil the then current diagnostic criteria was due to the lack of development of other relevant clinical signs and symptoms. However, if we follow the new diagnostic criteria, this would also now be considered a probable case.

Obviously one of the limitations of this study is that the final classification was based on the clinical symptoms and signs at review. It was difficult to follow up on the evolvement of other signs, especially in cases that followed a longer duration of illness as no follow up visits. However, the key point is that in the above cases, the RT-QuIC test was positive before the patient developed the required signs as set out by the old diagnostic criteria and therefore made an earlier and more accurate diagnosis. These cases highlight the usefulness of RT-QuIC in routine clinical practice where conventional measures of diagnosis failed to support a diagnosis at an early stage despite the high level of clinical suspicion.

4.5 Timing of Investigation Results

Diagnostic delay in sCJD is well recognised. The more classic presentations are typically rapid and patients present at different stages of their illnesses. As we have previously discussed, the initial stages of the illness can be non-specific and initial efforts and preliminary investigations are concentrated on identifying a potentially reversible condition. In our centre, the median time between symptom onset and diagnosis is 3-4 months (Professor Richard Knight, personal communication). During the early stages of the illness, it may be that an alternative cause other than sCJD how is being considered a possibility at that time. In some circumstances, it can be reasonable to adopt a 'watch and wait approach' with the evolvement of more typical features of sCJD becoming more apparent later on in the illness. In addition, sCJD is in invariably fatal condition and providing a diagnosis that is as secure as possible is important.

The timing of investigations varies between patients depending on how they have presented. Although less specific for sCJD, in vivo tests such as MRI, EEG and CSF are still important in the work up of a patient suffering from a progressive neurological illness as they may offer an alternative diagnosis that could potentially be treatable. Newer bioassays such as RT-QuIC has been shown to offer a higher degree of diagnostic accuracy as demonstrated by the high sensitivity and specificity indicated by this study and several other larger studies as discussed. However, providing an earlier diagnosis where possible also relies on providing test results in a timely manner once they have been performed.

With regard to MRI and EEG, there were some cases in this study where these investigations were considered negative in local centres but upon review by the NCJDRSU, were considered positive. The timing of the lumbar puncture varied

between patients during the course of the illness. Delays in providing a result from the date of lumbar puncture were largely related to logistical delays in transferring the sample from other centres around the United Kingdom. In addition, lumbar punctures were often performed initially to exclude or look for alternative neurological diagnoses with sCJD not yet considered a possibility. During this study, it was not uncommon for samples to be sent at a later date for processing.

However, it was recognised that our 'turnaround time' for providing RT-QuIC results is slower than some centres in comparison to more recent studies. Since the onset of this study, further work has been published demonstrating updated versions (so-called second generation) of RT-QuIC that has resulted in an improved processing time and sensitivity. A recent study demonstrated that increasing the temperature of the reaction mixture, longer shaking intervals with higher shaking speeds results in a faster RT-QuIC reaction without compromising sensitivity. The most widely used version of RT-QuIC employed by many centres can process samples between 24-90 hours and this is the same in our centre. However, under these newer conditions, this time has been shown to be reduced to between 4-14 hours which is very promising for the future and would have the potential for this novel test to be more cost-effective and improve the efficiency of CJD surveillance if diagnoses can be made earlier[215]. However, more data is required in this area before firm conclusions can be made.

4.6 Development and Implementation of the test

4.6.1 Further development of RT-QuIC

In addition to evaluating the measures that improve the speed of the RT-QuIC reaction, there are other variables that can influence the test outcome. During this study, it became apparent that the CSF volume used in the reaction mixture influenced the test result. By using a CSF volume of 30µl, we found that there was greater differentiation between a positive and negative result. Therefore, all testing initially used 30µl of CSF and if this was negative, the test was repeated using 15µl.

In addition, we found that CSF contaminated with blood had the ability to produce a false negative result and this has also been reported in other studies. One recent study reported that contamination with blood cells may influence the RT-QuIC response. In routine diagnostic practice, between 5 and 10% of the CSF samples are contaminated with blood during the lumbar puncture. The findings of this study indicated that blood contamination higher than 1250 cells/µl may inhibit the RT-QuIC reaction. Levels below this were considered negligible. Fresh blood-contaminated samples (3 days) can be rescued by removing the red cells[205].

4.6.2 Implementation and Reproducibility

In order for a test to be implemented into routine clinical practice, it has to be demonstrated to be reproducible and undergo a comprehensive validation and standardisation. Although many centres have developed their own protocols regarding RT-QuIC based on original studies, a recent study reported the first formal

multi-centre assessment of RT-QuIC, the outcome of which influenced the revision of the EuroCJD criteria and subsequent implementation of the test as previously discussed.

110 prion disease patients were analysed and 400 control patients using the RT-QuIC method under various conditions. In addition, 'blinded' ring trials between different centres were performed to estimate reproducibility. The study reported a sensitivity of 85% and specificity of 99%. The multi-centre inter-laboratory reproducibility of RT-QuIC revealed a Fleiss' kappa value of 0.83 indicating almost perfect concordance between centres. Two ring trials were performed between different laboratories. The first was between two centres using a high number of samples (54 sCJD and 32 controls) and indicated a substantial agreement with a Fleiss Kappa score of 0.75. Despite the high concordance, it was aimed to also identify the optimal conditions and standard operating procedures for the RT-QuIC method. The assay precision depends on various factors including inter-laboratory variations in staff, protocols and substrates, sensitivity of the assay to CSF sample storage and shipping temperatures and different fluorescence readers. By minimising any variation, the second ring trial achieved almost perfect agreement with a Fleiss kappa score of 0.83 thus demonstrating a high reproducibility of the assay with improved standardisation. A general problem with biomarker assays is that the sensitivity of the biomarker proteins to storage and shipment conditions. For example, during shipment, samples are subjected to a variety of temperatures and following arrival, also frequently undergo cycles of thawing and freezing. This study demonstrated that the PrP^{Sc} seed in the CSF of sCJD patients resisted the variations subjected to it with no impact on sensitivity or specificity. In addition, the study investigated various operational parameters including short term CSF storage at different temperatures long term storage, repeated freezing and thawing cycles and contamination of CSF by blood on the RT-QuIC seeding response. Overall, the study consolidated the reproducibility and high stability of RT-QuIC across various CSF storage conditions with a remarkable sensitivity and specificity supporting the current evidence that RT-QuIC is a robust and novel diagnostic method for sCJD[205].

4.7 Non Cases

Those cases classified as non-CJD cases either based on clinical grounds (4.2S) or neuropathological grounds (4.3S) were negative for all relevant tests including RT-QuIC emphasising the high specificity of this new test.

4.8 Conclusions

This study has supported initial and new evidence that RT-QuIC is a highly sensitive and specific antemortem test for the diagnosis of sCJD. It has been demonstrated via a series of case vignettes that it has advantages over more conventional tests in cases where the diagnosis has been particularly challenging. In addition, following the revision of the EuroCJD criteria during the course of the study, cases that were previously classified as possible or uncertain, have now been re-classified as probable based on the RT-QuIC result or MRI imaging.

It has been demonstrated through this study and larger studies that RT-QuIC is positive in all molecular subtypes of sCJD. However, genotype may have an influence on the test result and it remains contentious as to whether RT-QuIC can provide a 'definite' answer on sporadic CJD. Moreover, unlike brain specimens, RT-QuIC whether performed using CSF or olfactory epithelium does not provide any information on PrP^{Sc} glycotype, distribution of lesions, pattern of deposition or associated pathologies such as additional dementia types. Therefore, neuropathological examination remains the definitive means of diagnosis at this present time. As with other CSF protein biomarkers, the in vitro conversion reaction exploited by RT-QuIC is significantly affected by the neurobiological heterogeneity of prions. Studies on nasal brushings have been encouraging, although further work is required in this area.

4.9 Future Research

The data period for this study was 18 months. However, data collection by the team at NCJDRSU regarding the diagnostic utility of RT-QuIC has been ongoing until 2017 which provides 5 years of data analysing the usefulness of RT-QuIC in routine clinical practice in our centre and these findings will be published in the future.

In addition, aggregation of misfolded proteins is also implicated in other neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. In vitro amplification studies have shown that, similar to PrP^{Sc}, these misfolded protein aggregates can seed the normal protein isoforms through prion-like processes of replication. In addition, the olfactory bulb has been shown to be an early site of involvement in both Alzheimer's disease and synucleinopathies. Therefore, it seems possible that the development of highly sensitive seeding assays of olfactory mucosa brushings may assist the diagnosis of other proteinopathies and is an area of ongoing interest and research[216, 217].

Appendix 1

Poster Presentation

Association of British Neurologists

Cardiff 2014

Association of British Neurologists

This is to certify that

Louise Davidson

was awarded the Charles Symonds prize for

Best Poster Presented by a Junior Neurologist

at

Association of British Neurologists Annual Meeting, 7-9 May 2014

Wales Millennium Centre, Cardiff

President

Honorary Secretary

The Diagnostic Utility of RT-QuIC for the Diagnosis of Sporadic Creutzfeldt-Jakob Disease

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Introduction

Sporadic Creutzfeldt-Jakob Disease (sCJD) remains the commonest type of CJD with an incidence of approximately 1 per million and accounting for approximately 80% of cases[1]. The exact infectious mechanism remains unknown. However, the most widely accepted theory is the 'protein hypothesis' initially suggested by Prusiner and later developed over the years. It has been proposed that prion diseases result from the post-translational change of a normally expressed protein, named the prion protein (PrP^C) into a disease associated form (PrP^{Sc})[2]. PrP^{Sc} is partially protease resistant and has the ability to self-propagate by inducing further PrP^C to undergo conformational change thus producing more PrP^{Sc}, a so-called 'seeding effect'[3]. PrP^{Sc} subsequently aggregates and deposits throughout the brain, producing the neuro-pathological hallmarks of prion diseases which includes spongiform change, neuronal loss and astrocytic gliosis[4].

Despite its rarity, the clinical presentation is well described and classically follows a characteristic course of rapid onset dementia with associated neurological decline that often includes cerebellar ataxia, myoclonus, eventually leading to a state of akinetic mutism and death. The process frequently occurs within a period of 4-6 months[5,6]. Atypical presentations are less common but are well documented in the literature[7,8].

Currently, there is not a disease-specific, non-invasive ante-mortem test available for the diagnosis of sCJD with post-mortem remaining the definitive means of diagnosis. Current diagnostic criteria rely on clinical presentation in association with the MRI, EEG and CSF 14-3-3 protein[9]. However, none of these investigations are specific for CJD[10,11]. Atypical presentations can be diagnostically challenging and there can be a delay in diagnosis which can be distressing for relatives. This identified a need for a disease-specific, reliable diagnostic test that can provide an earlier and more accurate diagnosis.

A recently developed assay called real time quaking induced conversion (RT-QuIC) exploits the seeded conversion of normal prion protein to the abnormal form and therefore detects disease-associated prion protein in the CSF. Based on recent evidence, it has been suggested that RT-QuIC has the potential to identify cases that conventional techniques such as CSF 14-3-3 may miss and potentially contribute to an earlier and more accurate diagnosis[12,13]. We present the preliminary findings of the utility of RT-QuIC in routine clinical practice and whether it has any added benefit over current pre-mortem diagnostic methods. a prospective analysis

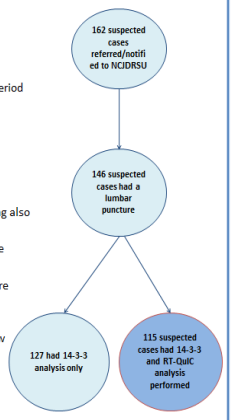
Methods

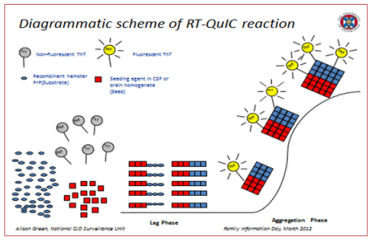
DATA SET

- 18 month data period
- Exclusion Criteria:
  - Non-UK cases
  - Referral outwith data period
  - Variant, genetic or iatrogenic case
- 192 suspected cases referred
- 30 excluded

ALL INCLUDED CASES n=115

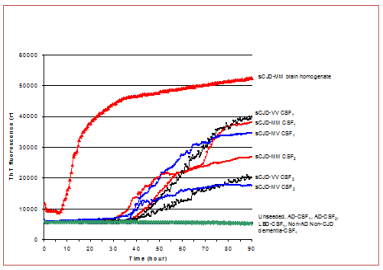
- All CSF received for 14-3-3 testing also tested for RT-QUIC
- Patient examined where possible
- Family interviewed using a standardised questionnaire where possible
- All cases classified according to WHO criteria pre and post review
- MRI and EEG independently reviewed where possible
- Genetic testing and codon 129 analysed following consent
- Brain tissue examined where possible





- CSF from a case of suspected sCJD is added to hamster recombinant PrP^C
- Seeded conversion of hamster recombinant PrP^C into aggregates of PrP^{Sc} is monitored in real time
- Thioflavin T (ThT) is included in the reaction and binds to the aggregated PrP^{Sc}
- This causes a change in the emission spectrum of ThT which is monitored using fluorescence spectroscopy

Typical RT-QuIC reaction curve for CSF samples from patients with sporadic CJD



- RT-QuIC reaction from sCJD brain homogenate starts within 10 hours and is maximal at 40 hours
- CSF reactions are slower with the RT-QuIC reaction starting between 25-45 hours and becoming maximal at 90 hours
- Reactions that are unseeded or seeded with CSF samples from patients with Alzheimer's disease (AD) or Lewy body dementia (LBD) remain flat during the time course of the RT-QuIC reaction

Findings

Case Classification (according to WHO criteria)	Total Number	RT-QuIC Positive	14-3-3 Positive	EEG Positive	MRI Positive (Basal Ganglia Change)	MRI Negative (but isolated cortical ribboning)
Definite	44	35 (80%)	36 (82%)	14 (32%)	24 (55%)	6 (14%)
Probable	45	38 (84%)	40 (89%)	14 (31%)	27 (60%)	4 (9%)
Possible	5	3 (60%)	0 (0%)	0 (0%)	0 (0%)	3 (60%)
Unknown	7	2 (29%)	2 (29%)	0 (0%)	3 (43%)	2 (29%)
Non Case-Clinically	7	0 (0%)	2 (29%)	0 (0%)	0 (0%)	0 (0%)
Non Case-pathologically	4	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)

Definite Sporadic Cases	RT-QuIC	14-3-3	EEG	MRI Basal Ganglia Change	MRI Isolated cortical ribboning
Sensitivity	80%	82%	32%	55%	14%
Specificity	100%	82%	91%	100%	
Positive Predictive Value	100%	95%	93%	100%	

- In concurrence with current literature, RT-QuIC is similar in sensitivity to 14-3-3 but more specific and has a greater positive predictive value
- It is considerably more sensitive than the EEG and also more specific
- It is more sensitive than MRI (Basal Ganglia change) but has equal specificity and positive predictive value
- Even when combining isolated cortical ribboning and basal ganglia change on MRI, RT-QuIC is still more sensitive
- RT-QuIC was negative in all of the non-cases. Negative predictive values could not be calculated as this study concentrated on REFERRED suspected cases and so numbers of non-cases in this context is small and would therefore give an inaccurate value.
- The 3 cases classified as 'possible' and RT-QuIC positive were highly suspected to be cases but died without post-mortem or are still alive.
- The 2 cases classified as 'unknown' and RT-QuIC positive were both clinically suspected as cases but died without post-mortem.

Conclusions

- Recent literature has demonstrated that RT-QuIC can diagnose sporadic CJD with a high degree of sensitivity and specificity
- This study supports this data
- In comparison to the other investigations used in the WHO diagnostic criteria, it shows the greatest sensitivity but equal specificity to MRI
- It has potentially picked up cases that have presented atypically but this cannot be proven without post-mortem examination, which is one of the limitations of this study.
- We appreciate that it is not always possible to perform a lumbar puncture in suspected cases of CJD but on a similar note, it could be particularly useful in cases where a MRI is not possible (e.g. due to patient agitation or pacemaker).
- Overall, this is a highly sensitive, specific test for the diagnosis of sporadic CJD. We propose that it is a useful adjunct to the current investigations employed by the WHO criteria, especially in cases where the diagnosis can be difficult.

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Appendix 2

NCJDRSU Surveillance Questionnaire

CLINICAL AND EPIDEMIOLOGICAL REVIEW

SECTION 1

GENERAL INFORMATION

1.

IDENTIFICATION INFORMATION

ID Number: ☐☐☐☐☐

1.1 What is *subject's* name?

First name: _____

Surname: _____

Other name(s): _____

(if subject is female and is/has been married)

record *subject's* maiden name or previous married names if different from current surname

_____

1.2 Sex of *subject?* ☐

1=male

2=female

1.3 When was *subject* born? ____/____/_____ (dd/mm/yyyy)

1.4 What do you consider to be *subject's* ethnic origin? ☐

1=Caucasian

2=Mixed/multiple

3=Asian

4=Black/African/Caribbean

5=Other

1.5 What is *subject's* marital status? ☐

1=single 2=married 3=widowed 4=divorced 5=separated 6=cohabitating

1.6 What is *subject's* present home address?

(*if subject is deceased)* What was *subject's* last home address before s(he) became ill?

House and street: _____

Town: _____

County: _____

Postcode: _____

1.7 Name of *subject's* consultant: _____

1.8 Hospital address

Name of hospital: _____

Street: _____

Town: _____

Postcode: _____

Tel. number: _____

1.9 *Subject's* hospital record number: _____

1.10 *Subject's* NHS number: _____

1.11 Name of *subject's* GP Name/Practice: _____

Street: _____

Town: _____

Postcode: _____

Tel. number: _____

_____

1.12 Date of interview/examination: ____/____/_____ (dd/mm/yyyy)

1.13 Interview/examination performed by: _____

_____

1.14 What is your (*respondent's)* name?

First name _____

Surname _____

Other names(s) _____

1.15 What is your *(respondent's)* relationship to subject? ☐

If other, *specify:*_____

1=partner
2=son/daughter
3=nephew/niece
4=sibling
5=cousin
6=father/mother
7=subject themselves
8=other

1.16 How long have you know *subject*? ☐☐☐☐

(record year since which *subject* known)

1.17 What is your *(respondent's)* address?

House and street _____

Town _____

County _____

Postcode _____

Telephone _____

1.18 Place of interview: ☐

1=hospital

2=home

3=other

SECTION 2

CLINICAL SURVEILLANCE AND EPIDEMIOLOGICAL REVIEW

BACKGROUND HISTORY

2.1 Surgical History

Surgical history questions refer to *subject's* history **BOTH BEFORE AND AFTER ONSET OF ILLNESS**

Has *subject* ever had any operations, including eye operations or stitching of wounds? ☐ 1= yes 2= not to respondent's knowledge

(If yes), record the year, hospital and type of operation:

1.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐
2.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐
3.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐

4.	Year: _____ Accuracy code (filled in at data entry stage) Name of Hospital: _____ Operation: _____ Group Category	☐ ☐
5.	Year: _____ Accuracy code (filled in at data entry stage) Name of Hospital: _____ Operation: _____ Group Category	☐ ☐
6.	Year: _____ Accuracy code (filled in at data entry stage) Name of Hospital: _____ Operation: _____ Group Category	☐ ☐

7.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐	
8.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐	
9.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐	
10.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐	
11.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ ☐	

	Group Category	
12.	Year:	Accuracy code (filled in at data entry stage)
	Name of Hospital:	
	Operation:	Group Category
	Record the total number of operations	Total no.of fracture operations (88=N/A 99=N/K) 88=not applicable

2.2 Organ or Tissue Transplant

Has *subject* ever received an organ or tissue transplant, including corneal or bone marrow transplant?

(If yes), record year hospital and organ/tissue(s) received: ☐ 1=yes

2=not to respondent's knowledge

Hospital	Organ:
Hospital	Organ:

	1=yes 2=not to respondent's knowledge	If yes, record year If no, record 8888
Cornea	☐	☐☐☐☐
Bone Marrow	☐	☐☐☐☐
Kidney	☐	☐☐☐☐
Liver	☐	☐☐☐☐
Other	☐	☐☐☐☐

2.3 Previous Medical History

All further questions in this section refer to *subject's* history **PRIOR TO THE ONSET** of the current illness

Does the *subject* have a record of previous hospital admissions EXCLUDING surgery AND unrelated to the present illness? ☐ 1= yes 2= not to respondent's knowledge

(If yes,) on how many occasions has the *subject* been admitted to hospital (excluding surgery)? ☐☐ 88=not applicable

(If yes), record the hospital name, the date(s) of admission and the reason(s) for admission:

Hospital: _____

Date of Admission: _____

Reason: _____

Hospital: _____

Date of Admission: _____

Reason: _____

Hospital: _____

Date of Admission: _____

Reason: _____

2.3 Previous Medical History (cont'd)

Hospital: _____

Date of Admission: _____

Reason: _____

Hospital: _____

Date of Admission: _____

Reason: _____

Hospital:

Date of Admission:

Reason:

Total no.of fracture admissions

(non-surgical) 88=N/A 99=N/K)

2.4 **Blood donation**

Has *subject* ever been a blood donor?

1=yes

2=not to respondent's knowledge

If yes, date(s) and place(s):

2.5 **Blood/blood product transfusion**

Has *subject* ever received a transfusion of blood or blood products?

For suspect cases do not include any transfusions related to current illness

If yes, give year, hospital and reason:

1=yes

2=not to respondent's knowledge

Year:

Hospital:

Reason and product (if known)

2.5 **Blood/blood product transfusion (cont'd)**

Year:

Hospital:

Reason and product (if known):

Year:

Hospital:

Reason and product (if known):

2.6 Has *subject* ever been diagnosed with an inherited bleeding disorder or primary immunodeficiency disease (eg haemophilia, Von Willebrand's)

☐ 1=yes

2=not to respondent's knowledge

If yes, give year, hospital and type of illness:

Year:

Treating hospital:

Type of illness:

2.7 Course of Injections

Has *subject* ever received a treatment involving a course of injections, for example, human growth hormone, human gonadotrophin, insulin, fertility treatment?

For suspect cases and controls do not include treatments related to current illness

(If yes), record year, name of therapy, frequency and reason): ☐ 1=yes

2=not to respondent's knowledge

Year:

Therapy:

Frequency:

Reason:

Year:

Therapy:

Frequency:

Reason:

Year:

Therapy:

Frequency:

Reason:

Year:

Therapy:

Frequency:

Reason:

2.8 Has *subject* **ever been notified** that they are at increased risk of CJD?

☐ 1=yes

2=not to respondent's knowledge

Risk category:

☐ 1=inherited prion disease (ie have been tested or a blood relative tested and have a mutation or two or more blood relatives with prion disease;

2=recipients of blood components from vCJD donors;

3=donors of blood components to people who develop vCJD;

4=other recipients who received blood from donors to vCJD cases;

5=bleeding disorders who received UK sourced clotting factors;

6=plasma product recipients (non-bleeding disorder patients);

7=highly transfused;

8=human derived hormone recipients, ie growth hormone or gonadotrophin;

9=dura mater graft

10=surgical contacts of CJD cases;

88=not applicable

Please give details _____

2.9 Has *subject* ever been diagnosed with diabetes or bowel disease? ☐ 1=yes

(If yes, please give details) 2=not to respondent's knowledge

☐ 1=inflammatory bowel disease;

2=insulin-dependant diabetes;

3=other;

4=other bowel disease;

8=not applicable

2.10 Family history

Pedigree (indicating years of birth and death)

Subject's **grandparents**

(including names and dates of birth)

Subject's **parents** and **parents' siblings**

(including names and dates of birth)

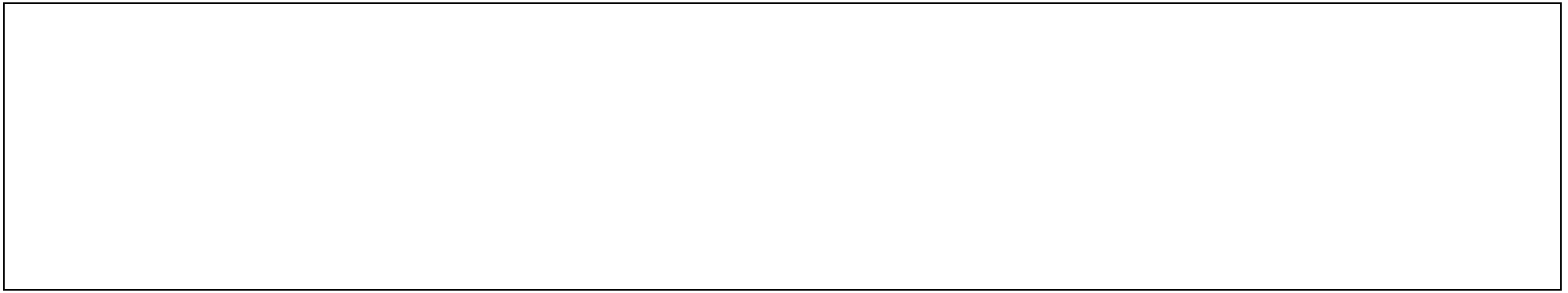
Subject and **siblings**

(including names and dates of birth)

Subject's children

(including names and dates of birth)

The Diagnostic Utility of RT-QuIC for the Diagnosis of Sporadic Creutzfeldt-Jakob Disease



2.11 Family History

Have any of the blood relatives of the *subject* included in the pedigree above died with dementia (or remain alive with dementia)?

☐ 1=yes
2=not to respondent's knowledge
3=respondent unsure

Have any of these individuals been diagnosed as having Creutzfeldt-Jakob disease?

☐ 1=yes
2=not to respondent's knowledge
3=respondent unsure

(If yes), record the person's name and the approximate date of illness:

Name: _____

Date of illness: _____

Confirmation of family history of CJD from surveillance database

☐ 1=definite case 4=unable to confirm
2=probable case 5=not a case
3=possible case 8=not applicable

2.12 Social Contact

Has *subject* had social contact, through family, friends or work, with someone else who developed CJD?

☐ 1=yes

2=not to respondent's knowledge

3=respondent unsure

(If yes), record the person's name and the approximate date of illness:

Name: _____

Date of illness: _____

Confirmation of social contact with case of CJD from surveillance database

☐ 1=definite case 4=unable to confirm

2=probable case 5=not a case

3=possible case 8=not applicable

2.13 Dietary History

Has *subject* ever been a vegetarian for a period of one year or more?

☐ 1=yes;

2=not to respondent's knowledge

(If yes), during what period(s) was *subject* vegetarian, and did s(he) eat any meat or fish at all during this time?

_____

_____

_____

2.14 Education

How many years of full time education did *subject* complete? ☐☐

(including school, college and university)

2.15 RESIDENTIAL HISTORY (begin with the most recent and work backwards)

	From (dd/mm/yyyy)	To (dd/mm/yyyy)	Street Town County	Postcode	OS grid reference
1	/ /	/ /			E N
2	/ /	/ /			E N
3	/ /	/ /			E N
4	/ /	/ /			E N
5	/ /	/ /			E N
6	/ /	/ /			E N
7	/ /	/ /			E N

8	/ /	/ /									E							
											N							
9	/ /	/ /									E							
											N							
10	/ /	/ /									E							
											N							

2.15 RESIDENTIAL HISTORY (continued)

	From (dd/mm/yyyy)	To (dd/mm/yyyy)	Street Town County	Postcode	OS grid reference
11	/ /	/ /			E N
12	/ /	/ /			E N
13	/ /	/ /			E N
14	/ /	/ /			E N
15	/ /	/ /			E N
16	/ /	/ /			E N
17	/ /	/ /			E N

18	/ /	/ /									E							
											N							
19	/ /	/ /									E							
											N							
20	/ /	/ /									E							
											N							

2.16 Exposure to animals

Since the beginning of 1980, has *subject* worked, lived or stayed for more than one week on a farm? ☐

1=lived or worked

2=stayed

3=not to respondent's knowledge

(If lived, worked or stayed), which type of farm was it? ☐

1=arable

2=livestock

3=mixed arable and livestock

4=other

8=not applicable

Duration of stay? _____

If livestock, please specify type _____

_____

Has *subject* ever hunted deer? ☐

1=yes

2=not to respondent's knowledge

If so, give details of where and when

_____

_____

_____

2.17 Occupational history of *subject*

(NB if any categories below are coded 1, please complete further details on following page)

1=ever

2=never

medical (medical/paramedical/nursing/dentistry) ☐

☐ laboratory (diagnostic/pharmaceutical/animal/other research)

☐ animal farming/veterinary medicine

☐ meat industry

(butchers/abbattoir staff/slaughterhouse workers/renderers/workers handling MRM etc)

☐ hauliers/incinerator operators/landfill site workers/cleaning & waste disposal workers

☐ maintenance engineers (eg in abbattoirs, rendering plans, incinerators etc)

☐ other occupation involving animal products (eg leather worker)

☐ catering industry (professional)

☐ workers in zoos and circuses

The Diagnostic Utility of RT-QuIC for the Diagnosis of Sporadic Creutzfeldt-Jakob Disease

2.17 OCCUPATIONAL HISTORY OF SUBJECT (cont'd)

	From (dd/mm/yyyy)	To (dd/mm/yyyy)	Name of employer	Town	Description of work
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					

14					

SECTION 3
EXAMINATION REVIEW

	Examination from notes State of *subject* at admission/first examination by a neurologist
3.1	General Appearance:
3.2	Mental state/speech function:
3.3	Cranial nerves:
3.4	Motor system: Involuntary movements:
3.5	Sensory system:
3.6	Reflexes: primitive: tendon: plantar:

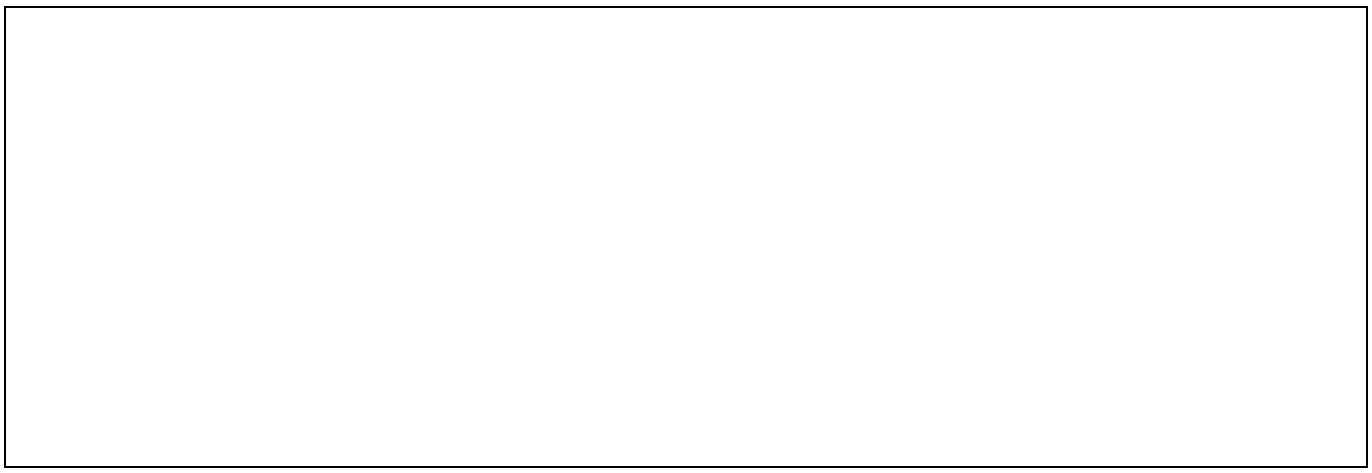
3.7	Cerebellar function/co-ordination
3.8	**NCJDSU Clinician – Examination of the *subject*** General Appearance: bed bound NG/PEG catheterised akinetic mute posture myoclonus startle other involuntary movements
3.9	Mental state/speech functions: best motor response best verbal response eye opening
3.10	Cranial nerves: fields/response to menace pupils EOMs/Doll's eyes
3.11	Motor system: tone power wasting

3.12	Sensory system
3.13	Reflexes primitive: grasp palmomental pout rooting tendon (incl. jaw jerk): plantar:
3.14	Cerebellar function/co-ordination:
3.15	General examination:

3.16 Detailed history of present illness:

3.16 Detailed history of present illness:

3.16 Detailed history of present illness:



CLINICAL HISTORY

3.17 Note the source of information eg hospital notes, relatives, other.

hospital notes ☐
relatives ☐
other ☐

1=yes
2=no
9=not known

If other, specify: _____

3.18 What were the first symptoms of illness noted by the *subject* or their family?

_____

3.19 When did these symptoms first occur? _____ (dd/mm/yyyy)

3.20 When did the *subject* seek medical attention for the illness? _____ (dd/mm/yyyy)

3.21 When was the *subject* first referred to a neurologist? _____ (dd/mm/yyyy)

3.22 When was the *subject* seen by a neurologist? _____ (dd/mm/yyyy)

3.23 When was the *subject* first admitted for the current illness? _____ (dd/mm/yyyy)

3.24 Since the start of the illness, has the *subject* been seen by a psychiatrist? ☐ 1=yes 2=no

3.25 If yes, record date of the first consultation: _____ (dd/mm/yyyy)

3.26 SPECIFIC ILLNESS DETAILS Since the start of the illness, until the current time, has the *subject* exhibited the following neurological symptoms/signs:

Symptoms:		**1=Yes 2=No 8=NK 9=Missing**	**Date appeared (dd/mm/yyyy)**	**Signs:**	**1=Yes 2=No 8=NK 9=Missing**	**Date appeared (dd/mm/yyyy)**
Forgetfulness/ memory impairment				Cognitive impairment		
Other higher function impairment				Agnosia		
				Apraxia		
				Visuospatial impairment		
Language disturbance				Dysphasia		
				Dyslexia		
				Dysgraphia		
Psychiatric symptoms				Grasp reflex		
Depression						
Anxiety						
Behavioural disturbance						
Apathy/withdrawal						
Delusions						
Hallucinations	Visual					
	Auditory					
Other psychiatric symptoms						
Disturbance of gait				Cerebellar gait ataxia		
				Spastic		
				Lower motor neurone		
				Other, specify		
Bedbound				Akinetic mutism		
Speech disturbance				Dysarthria		
Visual symptoms	Diplopia			Ocular motor palsy		
	Visual impairment			Hemianopia		
				Cortical blindness		

Weakness or clumsiness of limbs			Pyramidal weakness		
			Extrapyramidal signs		
			Cerebellar signs/nystagmus		
			Lower motor neurone		

Symptoms:	**1=Yes 2=No 8=NK 9=Missing**	**Date appeared (dd/mm/yyyy)**	**Signs:**	**1=Yes 2=No 8=NK 9=Missing**	**Date appeared (dd/mm/yyyy)**
Increased limb tone			Rigidity		
			Spasticity		
			Gegenhalten		
			Hyperreflexia		
			Extensor plantar responses		
			Muscle wasting		
			Fasciculation		
			Hypo- or Areflexia		
Involuntary movements			Myoclonus		
			Chorea		
			Dystonia		
			Other, specify		

Sensory symptoms	Numbness/tingling/ paraesthesia				**1=yes 2=no 3=central origin 4=peripheral origin 9=uncertain**	**Date appeared dd/mm/yyyy**
	Pain /burning / discomfort			Sensory signs		
Seizures						

3.27 TREATMENT

Specify drug therapy for TSE ☐

0=No treatment
1=Quinacrine
2=Oral PPS
3=Intra-ventricular PPS
4=Tetracycline (doxycycline)
5=Other, specify: _____

Complications of surgery ☐

1=yes

☐

Complications of intra-ventricular catheter		2=no 9=missing
Complications of pump	☐	
Significant drug side effects	☐	1=yes, withdrawal of drugs 2=yes, decrease of therapeutic dose 3=yes, but no change of the therapeutic dose 4=no 9=missing
Significant evidence of benefit	☐	1=clinical improvement 2=EEG improvement 3=MRI improvement 4=14-3-3 improvement 5=other improvement 6=no 8=Other, specify _____ 9=missing

SECTION 4
REVIEW OF INVESTIGATIONS

INVESTIGATIONS

4.1 EEG

Has the *subject* undergone an EEG?	☐ 1=yes; 2=no; 9=not known
(If yes), on how many occasions?	☐☐
(If yes), record date of most recent EEG	____/____/_____(dd/mm/yyyy)
Are EEG records/copies available in the Unit	☐ 1=yes 2=yes,some 3=no 8=not applicable
Have the EEGs been examined by a Unit staff member?	☐
Has the patient recorded an EEG characteristic of CJD (generalised triphasic periodic complexes with frequency about 1/s)	☐ 1=yes, confirmed by Unit staff 2=yes, reported by local staff, EEG not available for confirmation by Unit staff 3=no 8=no EEG performed
(If yes), record the date on which the first characteristic EEG was recorded	____/____/_____ (dd/mm/yyyy)

4.2 CT Scan

Has the *subject* ever had a CT scan?	☐ 1=yes; 2=no; 9=not known
Has the patient ever had an abnormal CT scan?	☐ 1=yes, confirmed by Unit staff 2=yes, reported by local staff, scan not available for confirmation by Unit staff 3=no 8=no scan performed
(If yes), record the date on which the first abnormal scan was performed?	____/____/_____ (dd/mm/yyyy)
(If yes), specify what abnormalities have been observed	_____ _____ _____

4.3 **MRI Scan**

Has the *subject* ever had an MRI scan? ☐ 1=yes; 2=no; 9=not known

(If yes), on how many occasions? ☐☐

(If yes), record date of most recent scan ____/____/_____(dd/mm/yyyy)

Are MRI scans available in the Unit ☐
1=yes
2=yes,some
3=no
8=not applicable

Have the MRI scans been examined by a Unit staff member? ☐

Has the patient ever had an abnormal MRI scan? ☐
1=yes, confirmed by Unit staff
2=yes, reported by local staff, scan not available for confirmation by Unit staff
3=no
8=no scan performed

(If yes), record the date on which the first abnormal scan was performed? ____/____/_____ (dd/mm/yyyy)

(If yes), specify what abnormalities have been observed _____

_____

_____

Please complete MRI results sheet on page 31 when scan has been reviewed at NCJDRSU

(If an abnormal MRI scan has been reported by someone outside the unit) who reported the abnormal scan?

Name: _____

Address _____

_____

_____

4.4 **CSF findings** (fill coding boxes with 8s if test results are not available)

Date of first CSF collection ____/____/_____ (dd/mm/yyyy)

Results:

protein ☐☐.☐☐ g/l

CSF glucose / serum glucose ☐☐/☐☐.☐☐ mmol/l

WBC / RCC ☐☐☐☐/☐☐☐☐ count/mm^3

14-3-3 ☐ 1=negative 2=equivocal 3=positive 4=bloodstained 5=not suitable/uninterpretable 6=weak positive

S100b ☐☐.☐☐ ng/ml

RTQuIC ☐ 1=negative 2=equivocal 3=positive 4=blood-stained 5=not suitable/uniterpretable

Ig oligoclonal bands in:

CSF ☐ 1=positive, 2=negative

blood ☐ 1=positive, 2=negative

Date of second CSF collection ____/____/_____ (dd/mm/yyyy)

Results:

protein ☐☐.☐☐ g/l

CSF glucose / serum glucose ☐☐/☐☐.☐☐ mmol/l

WBC / RCC ☐☐☐☐/☐☐☐☐ count/mm^3

14-3-3 ☐ 1=negative 2=equivocal 3=positive 4=bloodstained 5=not suitable/uninterpretable 6=weak positive

S100b ☐☐.☐☐ ng/ml

RTQuIC ☐ 1=negative 2=equivocal 3=positive 4=blood-stained 5=not suitable/uniterpretable

Ig oligoclonal bands in:

CSF ☐ 1=positive, 2=negative

blood ☐ 1=positive, 2=negative

4.5 Has the *subject* had neurophysiology studies done? ☐ 1=yes 2=no 9=not known

4.6 Has the *subject* had any abnormalities on other routine biological/haematological investigations during this illness? ☐ 1=yes 2=no 9=not known

(If yes), describe the investigation(s) and the abnormalities:

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

4.7 Has the *subject* undergone neuropsychological assessment? ☐ 1=yes 2=no 9=not known

Type of examination? ☐ 1=MMSE 2=Other, specify_____ 3=Formal Neuropsychometry 4= Multiple

Result

Score for MMSE _____

Score from other test (score)

_____

(please specify other test)

Is neuropsychology report available? ☐ 1=yes 2=no 9=not known

4.8 Has the *subject* undergone a brain biopsy? ☐ 1=yes 2=no 9=not known

(If yes), were there any complications? ☐ 1=yes 2=no 9=not known

Result _____

4.9 Has the *subject* undergone a tonsil biopsy? ☐ 1=yes 2=no 9=not known

(If yes), were there any complications? ☐ 1=yes 2=no 9=not known

Result _____

4.10 Specimens Collected

	1=yes 2=no	Quantity (mls)
Blood: Frozen for general use	☐	☐☐
Separated and frozen	☐	☐☐
Urine	☐	☐☐
CSF	☐	☐☐

4.11 Post Mortem

Date of death: ____/____/_____

Was a post mortem performed? ☐ 1=yes 2=no 9=not known

(If yes), is neuropathological material available? ☐ 1=yes 2=no 8=not applicable 9=not known

(If material is available) is material available in Edinburgh? ☐

Are post mortem results available? ☐ 1=yes 2=no 9=not known

4.12 PrP Genotype

Are PrP genotype data available? ☐ 1=yes 2=no 9=not known

(If yes), what was the ***subject's*** codon 129 genotype ☐ 1=MM 2=MV 3=VV

(If yes), did the subject carry a mutation? ☐ 1=yes 2=no 8=not done 9=missing

(If mutation), specify: _____

4.13 **Subject Classification**

On the basis of the available information, what is the classification of the *subject*? ☐☐.

1.0=definite CJD
2.0=probable CJD
3.0=possible CJD
4.1=diagnosis unclear
4.2=CJD thought unlikely
4.3=definitely not CJD
5.0=GSS
0.0=unclassified

(If subject is classified as at least possible CJD or GSS) which category of disease is suspected? ☐

S=sporadic CJD
N=variant CJD
G=genetic/familial CJD
I=iatrogenic CJD
G=GSS

Presenting symptoms ☐

1=rapidly progressive dementia
2=Heidenhain
3=Pure psychiatric onset
4=progressive dementia
5=pure cerebellar onset
6=stroke-like onset
8=other, specify: _____
9=missing

MRI DATA SHEET

Patient name: _____

NCJDRSU number: _____

MRI date: _____

Format: CD / Films Films loaded: Yes / No

MRI sequences: T1/T2 ☐ C / S / A

Flair ☐ C / S / A

DWI ☐ C / S / A

Quality of scan: ☐ 1=excellent; 2=good; 3=average; 4=sufficient; 5=insufficient; 6=poor

		RIGHT		LEFT	
		FLAIR	DWI	FLAIR	DWI
Basal ganglia	Caudate				
	Putamen				
	Globus pallidus				
Thalamus	Anterolateral thalamus				
	Mediodorsal thalamus				
	Pulvinar				
Cortex	Cingulate gyrus				
	Insular region				
	Hippocampi				
	Frontal				
	Parietal				
	Temporal				
	Occipital				

SUMMARY

Pulvinar sign: Yes / No

Basal ganglia high signal: Yes / No

Number of areas of cortical high signal _____

OVERALL IMPRESSION

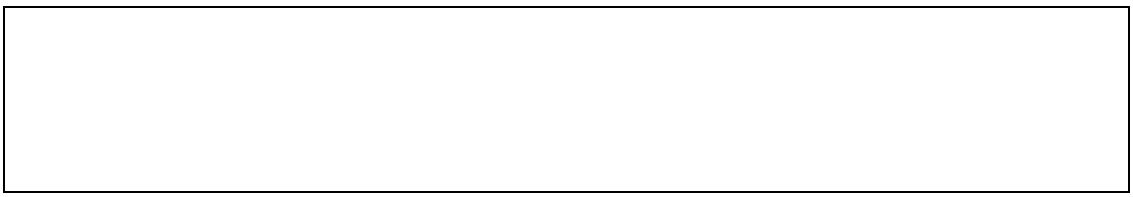
Inadequate scan ☐

Not suggestive ☐

Suspicious but not diagnostic ☐

Positive scan ☐

Other findings:



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